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Office of the Gene Technology Regulator

The biology of *Stenotaphrum secundatum* (Walter) Kuntze (buffalo grass)

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

Cover photo of buffalo grass courtesy of Feedipedia.

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ABBREVIATIONS

Abbreviation	Full name
APVMA	Australian Pesticides and Veterinary Medicines Authority
cm	centimetres
DNA	deoxyribonucleic acid
dSm^{-1}	decisiemens per metre
GM	genetically modified
IP	intellectual property
kg	kilograms
km	kilometres
m	metres
MCPA	2-methyl-4-chlorophenoxyacetic acid (a herbicide)
mm	millimetres
mM	millimolar
NSW	New South Wales
QLD	Queensland
spp.	species (plural)
syn.	synonym
USA	United States of America

PREAMBLE

This document describes the biology of *Stenotaphrum secundatum* (Walter) Kuntze (syn *Ischaemum secundatum* Walter), and its influence on the use and cultivation of *S. secundatum* in the Australian environment. The information included introduces the taxonomy and origins of cultivated *S. secundatum*, as well as providing general descriptions of its morphology, reproductive biology, biochemistry and biotic and abiotic interactions. This document also addresses the potential for gene transfer to occur to closely related species. The purpose of this document is to provide baseline information about the parent organism for use in risk assessments of genetically modified *S. secundatum* that may be released into the Australian environment.

S. secundatum arrived in Australia as deck cargo on board the *SS Buffalo* in the 1840s, giving rise to its common name, buffalo grass. In the United States of America, where selections of naturalised types have been cultivated since the 1880s, the species is commonly known as St. Augustinegrass (or St. Augustine grass). Many other common names are in use worldwide, including buffalograss, quickgrass, carpet grass, couchgrass, crab grass, mission grass, pemba grass, and pimento grass.

Buffalo grass/buffalograss is also the common name for several other grass species, particularly *Bouteloua dactyloides* (Nutt.) Columbus, which is native to North America. For the purposes of clarification, the species described in this document is *S. secundatum,* and when discussed in its Australian context it will be referred to as buffalo grass.

S. secundatum is widely used in Australia and overseas as a turf grass. There is historic, and to a limited extent current, use of the species as a forage grass and a ground cover in orchards and plantations in the Pacific region.

S. secundatum is a perennial grass plant that spreads by branching stolons, and forms coarse-textured canopy or thatch. When mowed or grazed regularly, the plant forms a dense turf, and when established the tight canopy makes the plant highly resistant to weed infestation. It is also shade tolerant and grows better under shade than under full sun, making the species suitable for gardens and plantations.

SECTION 1 TAXONOMY

Stenotaphrum secundatum (syn. *Ischaemum secundatum* Walter) is classified within the grass family Poaceae, previously known as Gramineae, the subfamily Panicoideae, and the tribe Paniceae (Busey, 2003b; Cook et al., 2005).

The Panicoideae subfamily are mainly distributed in warm-temperate to tropical habitats. The members of this subfamily include commercially important species such as *Zea mays* (corn), *Saccharum officinarum* (sugarcane), *Sorghum bicolor* (sorghum) and *Pennisetum glaucum* (pearl millet).

The tribe Paniceae contains 84 genera, and *Stenotaphrum* spp. belong to the Cenchrinae subtribe. The Cenchrinae also contains globally widespread genera such as *Cenchrus* L. and *Setaria* L. that contain species used for food, fodder and bioenergy, as well as species that are considered invasive weeds (Soreng et al., 2015; Washburn et al., 2015).

The *Stenotaphrum* genus consists of seven species, of which *S. secundatum* is the most abundant, occurring on all continents except Antarctica (Sauer, 1972). Section 2 of this document, *Origin and cultivation,* presents the distribution and primary characteristics of the seven species in more detail.

Adaptive and morphological variations in *S. secundatum* have been attributed to chromosomal differences within the species (Sauer, 1972; Busey, 1995; Genovesi et al., 2009). *S. secundatum* has a base chromosome number of $x = 9$ and a range of ploidy levels (Genovesi et al., 2009; Genovesi et al., 2017). The most common are diploids with 18 chromosomes ($2n = 18$) but triploids ($2n = 3x = 27$), tetraploids ($2n = 4x = 36$) and hexaploids ($2n = 6x = 54$) have also been reported, as well as aneuploids with 28, 30, and 32 chromosomes (Genovesi et al., 2017).

Sauer (1972) described fertile diploid plants within the species as the 'normal' race of the species, of which naturalised populations are found along coastlines of continents and islands of the Atlantic and Pacific oceans. Sauer (1972) also described two further races or 'demes[1]' within the species: the Cape deme (a sterile triploid) and the Natal-Plata deme (a fertile diploid), both originating in southern Africa.

A range of genotypes of *S. secundatum* that are cultivated in the USA, were classified for better understanding of the variation within the species, and for the purposes of orientating future breeding programs (Busey et al., 1982; Busey, 1986). Based on morphological characteristics, diploid cultivars were divided into Breviflorus and Longicaudatus races (Busey, 2003b). The Breviflorus Race was further divided into the Gulf Coast and Dwarf groups. The Longicaudatus Race was considered to be the same type as the Natal-Plata deme described previously (Sauer, 1972).

Busey et al. (1982) designated sterile triploid genotypes, synonymous with the Cape deme, as the Bitterblue Group, named after the foundation genotype of the Florida sod industry. Another group of polyploid variants was designated as the Roselawn-Floratam Group. Four genotypes were classified into a Miscellaneous Group, and these were similar to the Dwarf Group but thought to be inter-group hybrids (Busey et al., 1982).

[1] A local population made up of closely related organisms and large enough to have evolutionary significance

In Australia, there is lack of clarity about the genetic background of commercial cultivars of *S. secundatum.* Generally, long-established old-style buffalo grass lawns are most likely clones of the sterile Cape deme, introduced to Australia from the 1840s onwards (Sauer, 1972), which became known in Australia as 'common' buffalo grass (Loch et al., 2009). Cultivars selected and propagated for turf production in Australia are potentially selections of variants of the fertile normal race that has naturalised around Sydney and along the north coast of New South Wales (Loch et al., 2009).

SECTION 2 ORIGIN AND CULTIVATION

2.1 Centre of diversity and domestication

The centre of diversification for the *Stenotaphrum* genus is generally reported as the Indian Ocean region (Sauer, 1972; Busey, 1995), followed by transoceanic dispersal.

There are seven recognised species of the genus (Table 1), some of which are rare and endemic to particular tropical islands, while others are wide-ranging coastal pioneers (Sauer, 1972). The most abundant species is *S. secundatum*, which occurs on all continents except Antarctica. It is naturally and widely distributed as a seashore pioneer along the Atlantic coasts of the Americas and Africa (Sauer, 1972). More specifically, the centre of diversity for the species has been suggested to be coastal south eastern Africa, with the Gulf of Mexico and Caribbean region as a possible secondary centre (Beard, 2012).

Table 1. The life cycle, natural distribution and cultivation status of *Stenotaphrum* species. Adapted from Sauer (1972), Busey (2003b), and Busey (1995).

Species	Life cycle	Natural distribution	Present in Australia?	Cultivation status
S. clavigerum	Annual	Indian Ocean (endemic to Aldabra and Assumption Islands)	No	Not cultivated
S. dimidiatum	Perennial	Indian Ocean (east Africa and Madagascar to Sri Lanka)	No	Cultivated
S. helferi	Perennial	Southern China to Malaysia	No	Not cultivated*
S. micranthum	Annual	Indian Ocean to South Pacific	QLD	Cultivated
S. oostachym	Perennial	Endemic to Madagascar	No	Not cultivated
S. secundatum	Perennial	Worldwide, tropics to warm, humid regions	All states and territories	Cultivated
S. unilaterale	Perennial	Endemic to Madagascar	No	Not cultivated

*Although no deliberate planting has been reported, the species is used as pasture where it grows naturally (Sauer, 1972).

While the inflorescence of *S. secundatum*, as with other *Stenotaphrum* species, has a structure that enhances flotation on ocean currents, it will become waterlogged and sink within a week or so. The study of Sauer (1972) concluded that dispersal along shorelines and between islands was possible, however, the mechanisms for long-range, transoceanic dispersal were not completely clear. While human dispersal may have contributed to the range of the species, this is not supported by botanical records.

The fertile 'normal' race was recorded in botanical explorations as a coastal pioneer species on both sides of the Atlantic Ocean, in tropical West Africa, and the Americas from the Carolinas of the USA to Argentina before 1800. By 1840 it was in the Pacific region in Hawaii, Australia (including Norfolk Island) and New Zealand (Sauer, 1972). On several continents, the 'normal' race was transported inland and used as lawn grass but also became a local weed (Sauer, 1972). *S. secundatum* has been recorded in North America since the 1700s (Rosenberger and Busey, 1992) but it is not known whether it arrived on the continent during settlement by Europeans or before. The presence of a normal race of *S. secundatum* in Australia was attributed to its introduction by European settlers (Loch et al., 2009).

The Natal-Plata deme originated from the Natal region of South Africa and has been recorded throughout coastal Africa, temperate South America and south western Europe (Sauer, 1972).

The Cape deme originated from the Cape of Good Hope region in South Africa (Sauer, 1972). This variant was transported from South Africa throughout the nineteenth century and became naturalised in Australia, various Pacific islands and North America. The Cape deme was recorded in lawns, pastures and along coastal regions, in all Australian states over the period 1911 to 1955 (Sauer, 1972). In Australia, it is known as 'common' buffalo grass reflecting the introduction of the Cape deme during the 1840s, as deck cargo on board the *SS Buffalo* (Loch et al., 2009).

It was proposed that the Cape deme of the species replaced the normal race of *S. secundatum* throughout the Australasian region, as well as in Hawaii and California (Sauer, 1972). However characterisation of commercial and naturalised genotypes across Australia (Loch et al., 2009) concluded that a variable population of normal *S. secundatum* remained along the New South Wales mid-north coast, with its centre of diversity potentially located in the lower Hunter Valley. These populations were identified as the main source of genetic material for 'soft-leaf' buffalo grass cultivars that have been developed for Australian turf markets since the 1970s (Loch et al., 2009).

2.2 Commercial uses

The modern commercial use of *S. secundatum* in tropical, subtropical, and warm temperate regions of the world is as a turf species for public and private property; in particular, it is a commercially important species for the production of ready-to-lay turf or sod (Busey, 2003b; Loch et al., 2009).

S. secundatum was first recorded as being planted for turf grass in Florida, USA, in the 1880s and the commercial production of sod for establishing lawns in the region was underway in the 1920s (White and Busey, 1987). Since the 1970s, more critical selection of parent material has been made from local populations in Australia and the USA to develop cultivars for specific regions and end uses (White and Busey, 1987; Loch et al., 2009).

Historically, *S. secundatum* was used as a pasture species in the tropics and subtropics (Busey, 1995), however the species has been superseded by more productive species in high productivity agricultural systems. In Australia, buffalo grass is no longer recommended for pasture improvement and in the USA, the species is no longer the subject of forage research (Mullen and Shelton, 1996).

In the Pacific islands, *S. secundatum* was used as a forage and cover crop in plantation crops from the late 1800s (Sauer, 1972) and its ability to maintain yield and vigour in the shaded environment of plantations continues to be recognised (Mullen and Shelton, 1996; Smith and Valenzuela, 2002).

Buffalo grass is well suited to use as a cover crop as it suppresses weeds by blocking light and outcompetes weeds for water and nutrients. Reduced disease outbreaks and improved crop quality of tree crops are also reported with buffalo grass cover crops (Mullen and Shelton, 1996).

The growth habit of buffalo grass is well-suited to stabilising vegetation for sandy and fragile soils. For example, buffalo grass was imported from the USA in the early 20th century to revegetate cleared sand dunes on the Kurnell Peninsular in the Sydney region, now known as the Cronulla Sand Dune (Office of Environment & Heritage, 2017); and it was introduced to Montague Island in 1916 to establish lawns around buildings and prevent further erosion of sandy soils (Heyligers and Adams, 2004).

In recent and current times, buffalo grass has been regarded as a desirable species for roadsides and open public spaces, primarily due to its ability to minimise weed invasion (Navie et al., 2010) but also for its greater tolerance of wear compared with other warm-season turf grasses (Duff et al., 2009). Buffalo grass possesses moderate salt tolerance (Loch et al., 2006), and higher tolerance of shade than other comparable grass species, such as kikuyu (*Pennisetum clandestinum*) and couch grass (*Cynodon dactylon*) (Loch et al., 2006).

2.3 Cultivation in Australia

Buffalo grass is a commercially important species in Australia (Horticulture Innovation Australia, 2017), where the cultivation of buffalo grass is almost exclusively for the purposes of producing turf (Cook et al., 2005; Loch et al., 2009). The grass is not grown for seed production nor is it grown for grazing purposes (although it may be present as a volunteer in some pastures). Turf production, including buffalo grass, occurs in all states and territories of Australia but predominantly in Queensland and New South Wales (Horticulture Innovation Australia, 2017).

Due to its growth by stolons[2], buffalo grass maintains good ground cover and can spread relatively rapidly compared with lawn species that establish by seed or seedlings (Aldous et al., 2014). It provides excellent competition against weeds of lawn areas by forming a dense thatch. Compared with other turf grasses, such as kikuyu and couch grass, buffalo grass is less invasive in gardens. In part, this is due to the fact that buffalo grass spreads by stolons only, compared with kikuyu and couch grass that spread by stolons and rhizomes (Layt, 2017).

2.3.1 Commercial propagation

Due to its largely vegetative growth habit, the process of propagation and cultivation for commercial production of buffalo grass is the same. In turf cultivation, *S. secundatum* is propagated asexually by planting (or to a lesser degree, broadcasting) stolon cuttings, plugs or sods[3] (Macfarlane and Shelton, 1986; Genovesi et al., 2009; Aldous et al., 2014).

In Australia, commercially available buffalo grass is grown from segments of an established sward. When establishing buffalo grass for turf production, runners, plugs or sprigs are transplanted directly into the prepared seed bed (Buffalo Lawn Care). Once the field is established, the turf harvesting process leaves a proportion of the established turf behind, allowing regeneration. The main means

[2] A horizontal plant stem or runner

[3] Sod is mature turf grass that has been grown and cut into long strips by professional growers. Plugs are small sections of sod that have mature roots.

of supplying buffalo grass to the market is as rolls of turf or sod. As the rolls of turf are harvested from the ground, narrow strips are left between each roll. These strips will spread across the harvested area to form the new sward (Buffalo Lawn Care).

There is no commercial seed production of *S. secundatum* in Australia, such as seed for selling in garden centres[4]. Even though some cultivars developed from naturalised and imported populations produce viable seed (Loch et al., 2009), seed production does not warrant commercial investment when vegetative propagation is well established and successful (Aldous et al., 2014).

2.3.2 Scale of cultivation

Growing requirements

S. secundatum is a perennial, warm-season species with a C4[5] photosynthetic pathway. It grows best at between 20 °C and 30 °C, and the minimum temperature for growth is 10 °C (Cook et al., 2005). Cold and frost tolerance varies with ploidy level and cultivar. Diploid types are more tolerant of cold and frost than other C4 grasses, and have better cool season growth and winter survival than triploid and polyploids (Cook et al., 2005).

Generally, buffalo grass grows actively when temperatures are sufficiently high and water is not limiting. In lower-altitude equatorial climates, year-round growth can be expected. In warm-temperate areas, growth is distinctly seasonal, slowing in the autumn and becoming dormant in winter (Aldous et al., 2014).

While *S. secundatum* is adapted to areas receiving 1000 mm to over 2000 mm of annual rainfall, it is able to establish in areas of 750 mm annual rainfall, if soil conditions remain moist (Cook et al., 2005). Despite its natural preference for high moisture environments, *S. secundatum* is moderately drought tolerant (Busey, 2003b; Cook et al., 2005). Experimental work in Australia has shown that buffalo grass has the ability to tolerate extended periods of water deficit and resume growth and regain greenness when water supply is restored (Duff et al., 2009; Colmer, 2012). Polyploid cultivars exhibit greater drought survival than diploids, and there is variation in the extent of drought survival between cultivars (Busey, 2003b).

The preferred soil pH range for buffalo grass in Australia is 5.0–7.0 and it will grow across a range of soil textures (Duff et al., 2009). In the USA, it grows successfully on sand, loam and humic soils, ranging in pH from 4.5 to 8.5 (Busey, 2003b).

Buffalo grass does not have specific nutrient requirements for growth and the species is widely recognised as a pioneer of coastal environments (Sauer, 1972; Loch et al., 2009), where soil fertility is poor. While buffalo grass can withstand poor soil fertility, types selected for turf production and subsequent cultivation in public and residential places respond well to a balanced fertiliser program (Aldous et al., 2014). Nutrient management for buffalo grass production is discussed in Section 2.3.3.

[4] An internet search (via Google) of lawn seed products available at Australian hardware or nursery outlets identifies a buffalo seed product however this is referred to as North America buffalo grass (McKays, 2017), a different species to Australian buffalo grass — potentially *Bouteloua dactyloides* (syn. *Buchloe dactyloides*) or *Paspalum conjugatum*.

[5] C4 carbon fixation is one of three widespread biochemical processes, along with C3 and CAM photosynthesis, used by plants to fix carbon dioxide. It is named for the 4-carbon molecule present in the first product of carbon fixation. It is less common than C3 photosynthesis.

Production areas

Turf production, including buffalo grass, occurs in all states and territories of Australia but predominantly in Queensland and New South Wales, with these two states accounting for 38% and 33% of turf produced, respectively. In Queensland, the major turf production areas are the Cairns, Sunshine Coast, Wide Bay, Lockyer Valley and Scenic Rim regions; and in New South Wales, the major areas are the Lower Hunter, Central Coast and Hawkesbury regions (Horticulture Innovation Australia, 2017). A range of cultivars are produced in these regions. In Victoria and Western Australia, turf production is mainly cool-season turf species, however a limited number of buffalo grass cultivars are produced in the Greater Melbourne and Greater Perth regions (Turf Australia).

In 2015–16, turf production of warm-season and cool-season species was 47.2 million square metres, from almost 4500 hectares. Production was entirely for the domestic market, with a wholesale value of A$314 million (Horticulture Innovation Australia, 2017). There were 235 farm businesses producing turf in the 2014–15 year (ABS, 2016).

Buffalo grass accounted for 33% of the total production of turf in Australia in 2015–16 (Horticulture Innovation Australia, 2017).

Production areas are defined not only by the growing requirements identified in the previous section, but also by physical land characteristics and proximity to markets. Turf farms are generally located on flat land with fertile soils, typically on the floodplains of waterways (Turf Australia, 2016). Turf farms are often located close to urban centres, minimising the time between supplier and customer, which is important for quality control of the product.

Cultivars/varieties in use

Naturalised and introduced forms of buffalo grass in Australia have been selected and propagated to establish recognised varieties (Duff et al., 2009; Loch et al., 2009). Details of registered varieties are available in the Plant Breeder's Rights database of IP Australia. A number of cultivars developed in the USA are also commercially available in Australia (Loch et al., 2009).

Given the morphological and genetic diversity with the species *S. secundatum,* the differences between cultivars are potentially significant, which is reflected in the range of growing areas of buffalo grass in Australia, from tropical north Queensland to cool temperate Victoria Australia (Loch et al., 2006; Duff et al., 2009; Loch et al., 2009).

At the time of writing, there were no Australian cultivars that had been developed using genetic modification.

2.3.3 Cultivation practices

There are three main end-points for the cultivation of turf grasses:

- production of sod for the 'ready-to-lay' turf market
- provision of utility, aesthetics and ground cover in public places
- provision of aesthetics and ground cover for home gardens.

While cultivation practices are similar for all end-points, there is a fundamental difference in that turf producers manage for maximum production and efficiency of inputs; whereas ground managers and home gardeners manage for optimal aesthetics (greenness and density) with less production (to reduce mowing requirements and management of clippings).

The following sections principally address the cultivation of buffalo grass for turf production; but if amenity or domestic cultivation differs significantly, those points of difference are also discussed.

Planting and growth

Planting times vary by variety and location. Generally, year-round growth is typical in lower-altitude equatorial climates, and seasonal growth is observed in warm-temperate areas of Australia, with growth slowing in autumn and ceasing in winter as the grass goes into dormancy (Aldous et al., 2014). Based on trials in Australia (Duff et al., 2009), and guidelines prepared for the general public (Buffalo Lawn Care), best results are likely when buffalo grass is established in late summer and autumn in northern Australia, and in spring in southern Australia.

Establishment of buffalo grass is very rapid (Kaligis et al., 1995), but newly sown plant material can take significant time to spread and establish a field of turf that is ready for harvest. The rate of spread will vary with planting rate, cultivar, environmental conditions, and management. At recommended establishment rates[6], a planting may take 5–6 months to cover the required area (Cook et al., 2005). Experimental work in southern Queensland recorded the greatest rate of growth of Australian buffalo grass cultivars with September sown plots, where plant diameters grew from 6 cm at planting to between 130 cm to 185 cm, 85 days after planting (Duff et al., 2009).

In the case of turf production, narrow strips of turf are left *in situ* during the harvest process, from which the sod can re-establish. Regrowth of saleable turf from these residual plants can take as few as three months in Australian conditions (LawnGreen), and one plot of turf may be harvested multiple times before the planting loses vigour or becomes unsuitable for turf production.

The rapid rate at which buffalo grass can regenerate after harvest (sod removal) has meant that vegetative methods of establishment remain the preferred method for cultivation (Aldous et al., 2014).

For turf production, buffalo grass is grown as a monoculture. It does not require other plants or species to be grown in association to aid nutrition, soil health or pollination. Further, the market for buffalo grass turf prefers a ‘pure’ stand of a single species.

Nutrition

Nitrogen is a key nutrient for the production of quality turf (Aldous et al., 2014). For instance, the cultivar ‘Sir Walter’ has an optimum nitrogen requirement of around 200 kg per hectare per year to retain acceptable colour (Duff et al., 2009). While applications throughout the year are required, an early spring application of a significant proportion of the turf’s total nitrogen requirement reduces peak summer growth, therefore reducing mowing requirements (Duff et al., 2009).

[6] 7–10 cm plugs planted at 60–70 cm apart or broadcasting sprigs at 3.5–7.0 m^3 per hectare

Other macro and micro nutrients should be applied to turf as required, based on regular soil or tissue tests (Aldous et al., 2014). However, this approach is limited by a lack of specific data on critical concentrations of nutrients in plant tissue for buffalo grass (Duff et al., 2009). Soil amendments such as organic matter (e.g. compost), lime, dolomite and gypsum should be applied in conjunction with a fertiliser program (Aldous et al., 2014).

In pasture production in tropical plantations, *S. secundatum* survives and maintains growth without fertiliser or under low levels of fertiliser application (Mullen and Shelton, 1996; Cook et al., 2005).

Irrigation

Irrigation of buffalo grass is essential for maintenance and growth, particularly in hot summer months, and to support commercially viable growth rates in commercial turf production (Aldous et al., 2014). Irrigation at 50% replacement of net evaporation over a period of 16 weeks during summer maintains buffalo grass in acceptable condition, however irrigation at 33% of net evaporation over the same period did not maintain acceptable condition (Duff et al., 2009). Buffalo grass plantings survived periods of water shortage and recovered when re-watered (Duff et al., 2009). Similarly, Colmer (2012) reported that buffalo grass (and other warm-season turf species) that were watered once a week during summer and subsequently turned brown, were able to recover when irrigation increased in autumn.

Management of weeds, pests and disease

Weed management of turf grass for commercial production is important to control and maintain product purity.

Field preparation before establishment of grass for turf production is a critical time to control perennial weed species and previous turf grass swards (Aldous et al., 2014). As with most crops, cultural management to encourage vigorous establishment and growth of the target plant also suppresses weed growth. Further, commercial turf grasses are mown frequently to encourage coverage, which prevents growth and seed set of most weed species (Aldous et al., 2014).

Non-chemical means of weed control involve maintaining adequate nutrient and water supply to the lawn, so that the buffalo grass remains competitive, and controlling disease and pests to prevent bare patches occurring (Aldous et al., 2014). In general, buffalo grass is better at out-competing weeds than turf varieties like kikuyu, couch grass, and fescue (Layt, 2017), however other stoloniferous and rhizomatous lawn species such as kikuyu and couch grass may infest a poorly growing buffalo grass lawn.

Herbicides may sometimes be required to control broadleaf and grass weeds in buffalo grass. Some herbicides can be used safely on buffalo grass to control target weeds but there are herbicides that will cause plant injury to buffalo grass, although they are safe for use on several other lawn or turf species (Layt, 2017). In Section 6.2.3, Table 3 lists herbicide products that are registered for use for weed control in buffalo grass in Australia.

In lawns, regular renovation to remove the build-up of thatch is important to maintain the health and vigour of buffalo grass (Aldous et al., 2014)[7], as well as suppress weeds (Busey, 2003b; Aldous et al., 2014). Further, a very dense layer of thatch can reduce the effectiveness of herbicide application, if there is a weed incursion, by absorbing the product before it reaches the target plant (Aldous et al., 2014). The development of a thick thatch provides harbour for disease pathogens and insect larvae, therefore regular renovation is important to minimise incursions (Colmer, 2012; Aldous et al., 2014)

See Section 7.1 for further discussion about potential weeds of buffalo grass.

Pests and diseases can be minimised by maintaining a well fertilised and vigorous lawn (Aldous et al., 2014; Layt, 2017). However, certain conditions could facilitate a pest or disease outbreak. For example, in humid areas, disease pressure is greatly reduced if turf is watered in the morning rather than afternoon or evening (Layt, 2017).

Infection by pathogens can be controlled by management (better fertilisation and adjusting watering regimes). A range of pesticide and fungicide products are available for control of many diseases and pests (Aldous et al., 2014; Layt, 2017). Biological control of some insects is also possible with biopesticides such as those that contain the bacterium *Bacillus thuringiensis*.

Discussion about potential pests and diseases of buffalo grass is found in Section 7.2.

Harvest of turf and post-harvest management

Harvest of commercially cultivated turf involves cutting strips or slabs of the grass, and a shallow depth of underlying soil, from the field. The harvested turf is rolled or stacked, depending on harvest method, and aggregated onto pallets for transport to the customer or retail outlet.

Management of growth for amenity and domestic lawns

Where turf is cultivated to provide ground cover in public, commercial and residential spaces, mowing and disposal of clippings is a significant management cost (McCarty et al., 2004; Aldous et al., 2014) and water conservation is of increasing importance (McCarty et al., 2004; Colmer, 2012).

As well as breeding cultivars for reduced growth, the application of plant growth retardants is a means of reducing vertical and lateral growth, particularly in seasons of high rainfall or water supply. Use of growth retardants has been a successful strategy for managing a number of turf grass species in the USA (Weinbrecht et al., 1998), however research on *S. secundatum* is limited (McCarty et al., 2004).

A number of plant growth retardants are registered for use on buffalo grass in Australia (APVMA, 2017) however the use of retardants (or chemical growth inhibitors) is generally limited to sports stadiums, during periods of maximum growth, to minimise frequency of mowing (Aldous et al., 2014).

[7] Reduction of thatch: All warm season grasses will produce a layer of built up lawn runners (stolons) that will increase in thickness over time. Buffalo grass is known for increasing its base thickness more than other lawn grass varieties, easily growing to a thickness of 20 cm or more (Buffalo Lawn Care).

2.4 Crop improvement

Some deliberate selection of desirable types of *S. secundatum* has occurred since the early 20th century, however major improvements of the species for its use as turf grass has occurred mainly since the 1970s in the USA (White and Busey, 1987) and Australia (Loch et al., 2009). Improvement of buffalo grass for use as turf has almost exclusively been based on visual appraisal of phenotype and clonal propagation, thus there is limited knowledge regarding cultivar lineage and the full range of traits of many commercial cultivars in Australia and the USA (Busey, 1986; Casey Reynolds et al., 2009; Loch et al., 2009).

In an effort to improve *S. secundatum* by means other than selection and cloning, some researchers have investigated technologies such as induction of somoclonal variation and irradiation mutagenesis to generate new cultivars or new lines for selection in future cultivar development (Casey Reynolds et al., 2009; Li et al., 2010; Cakir et al., 2017) .

Embryo rescue techniques have been used to overcome the problem of aborted seed that often occurs with interploid crosses (Genovesi et al., 2009). These interploid hybrids potentially enable better and more complete use of genetic variation within the species, leading to new genetic combinations with a greater range of tolerance to biotic and abiotic factors (Genovesi et al., 2009). For example, a new cultivar registered in the USA in 2014, DALSA 0605, was the first embryo rescue-derived interploid variety of *S. secundatum* released for commercial cultivation (Chandra et al., 2015). The new cultivar produces non-viable seed, and exhibits a wide range of traits derived from its diploid and triploid parents, including drought, disease and pest tolerance.

2.4.1 Breeding and selection

In Australia, the development of buffalo grass cultivars has been described as “haphazard (one-off) selection of superior plants found among naturalised material or as contaminant seedlings on turf farms” (Loch et al., 2009). Two basic genotypic groups of *S. secundatum* exist in Australia, the sterile triploid Cape deme (common buffalo grass) and a normal fertile diploid race (established along the mid-north coast of NSW). Most of the development of commercial cultivars of buffalo grass has been based on selections from the latter group (Loch et al., 2009).

Historically, buffalo grass used for household and public lawns in Australia was propagated from runners of the introduced common buffalo grass. Shademaster was the first soft-leaf cultivar developed and commercialised in Australia, released in the 1990s (Buffalo Lawn Care). It was selected from a population of normal *S. secundatum* in the lower Hunter Valley of NSW (Loch et al., 2009). Many later cultivars either had Shademaster as parent material or were similarly selected from naturalised plant populations in the Hunter or Hawkesbury regions of NSW (Loch et al., 2009).

Improvements of buffalo grass in Australia have targeted superior physical characteristics such as colour and greater leaf to stem ratio. Loch et al. (2009) concluded that future commercial development of the species should focus on the capacity of buffalo grass to tolerate wear, management (especially mowing), varying levels of shade, water use and herbicide tolerance. The authors also identified the future challenge of developing varieties that meet the conflicting demands of turf producers and turf consumers. Producers prefer aggressively growing types that can be harvested frequently, whereas turf managers and home gardeners prefer less spreading types that require less mowing.

Some USA cultivars of *S. secundatum* have become established in Australia. These have been developed from “normal” diploid types in the USA, and show distinctly different DNA profiles compared with Australian-derived "normal" types (Loch et al., 2009). As with commercial cultivars in Australia, commercial cultivars in the USA have historically been produced by clonal propagation.

Breeding between different races of *S. secundatum* has been impeded by differences in ploidy levels. The perennial nature of *S. secundatum* has also limited breeding efforts, as long-term field evaluation is required to ensure exposure to environmental and biotic problems and for the plant to exhibit tolerances or sensitivities to these factors (Busey, 1995). There has been limited or no public expenditure on the improvement of *S. secundatum* in the US, compared with other turf grasses, as the species is predominantly used for lawns rather than golf or sports turf (Busey, 2003b; Cakir et al., 2017).

Recent characterisation studies highlight the benefits of gaining more knowledge of genetic relationships between groups and types of *S. secundatum*, in order to more effectively and strategically use germplasm across ploidy levels for cultivar development (Milla-Lewis et al., 2013; Mulkey et al., 2014).

2.4.2 Genetic modification

A number of turf grass species, particularly C3 plants but also the C4 plants *Zoysia japonica* (zoysia grass) and *Cynodon* spp. (Bermuda grass), have been genetically modified (Song et al., 2013a). This has been primarily to introduce herbicide resistance to species commonly used on sports fields, particularly golf courses, but several laboratories are also developing grasses that are tolerant to biotic or abiotic stress factors.

Techniques for genetic modification of buffalo grass have been developed (Lee and Berg, 1999; Torisky et al., 2005, 2007). The USA company Scotts-Miracle Gro has modified several turf grasses, including *S. secundatum,* for traits of glyphosate tolerance and slower growth (Waltz, 2015, 2018).

SECTION 3 MORPHOLOGY

3.1 Plant morphology

S. secundatum has a prostrate growth habit (Clayton et al., 2006) and grows and spreads by branching stolons, with no evidence of growth via rhizomes. The stolons are somewhat flattened and branches develop at nodes (i.e. joints) along the stolon (Figure 1A). Where the node is in contact with the soil, adventitious roots[8] may be produced, forming new plants. (Smith and Valenzuela, 2002; Busey, 2003b; Cook et al., 2005). *S. secundatum* forms a dense, coarse, spongy canopy, especially when mowed or grazed (Busey, 2003b).

[8] Roots forming from the stem or another plant organ, rather than from other roots, making it possible to vegetatively propagate a plant from a leaf or stem.

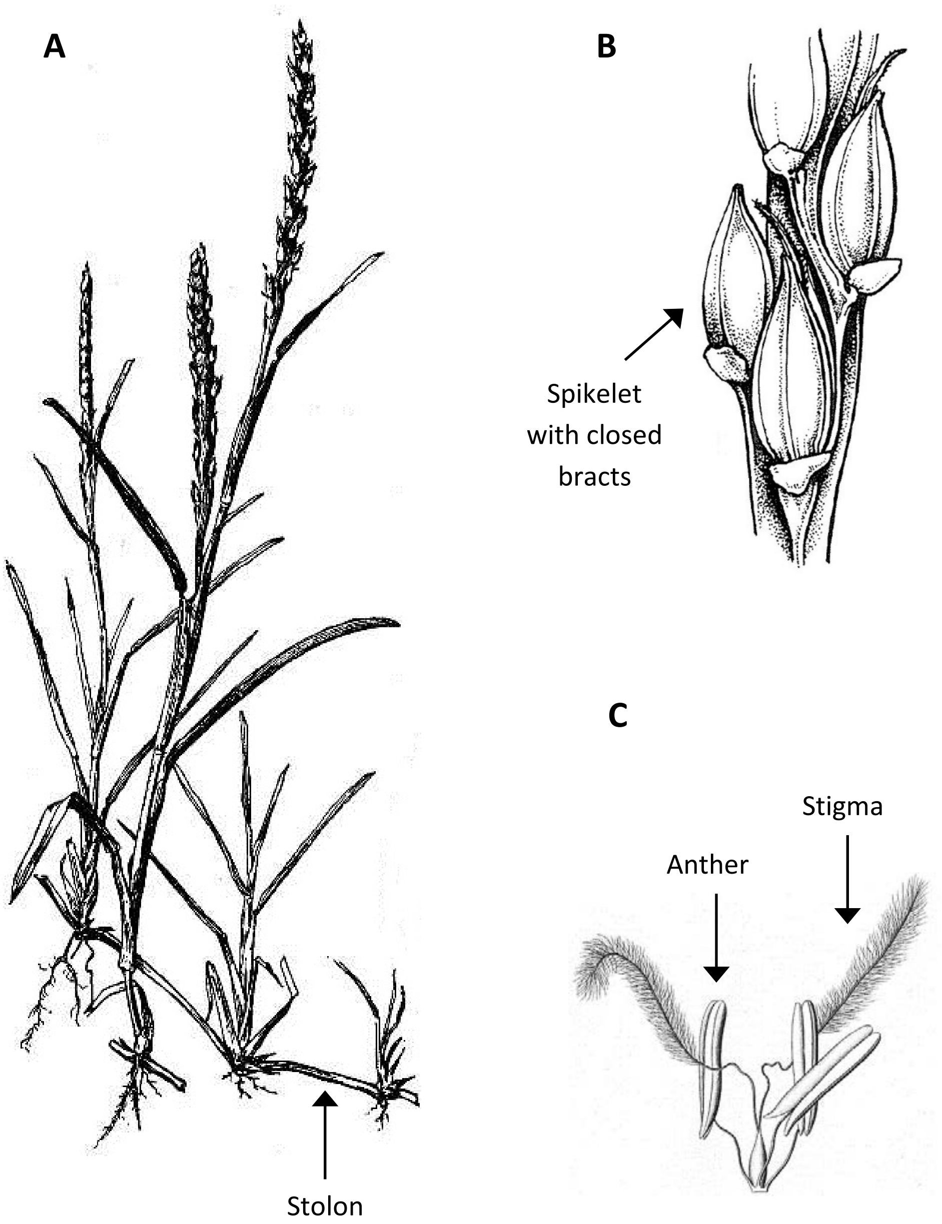

Figure 1. Morphology of buffalo grass. **A**: *Stenotaphrum secundatum* plant; adapted from Hitchcock (1935). **B**: Portion of *S. secundatum* inflorescence, courtesy of A. Barley, National Herbarium of Victoria. **C**: Detail of fertile floret from *S. dimidiatum*; adapted from Kunth (1835).

Leaves consist of a leaf sheath, which partially encloses the stem, and a spreading leaf blade. The leaf sheaths are 30–60 mm long, folded, usually pale green or whitish towards the base and mostly hairless, except for some long hairs near the base. The leaf blades are folded when young and may be flat or folded when mature. Leaf blades are 20–300 mm long and 30–140 mm wide, have smooth margins and, unlike most grasses, generally have rounded tips. Leaves are arranged alternately along the stems but may sometimes appear almost paired when the stem joints (i.e. nodes) are very close together (Smith and Valenzuela, 2002; Busey, 2003b; Cook et al., 2005; Biosecurity Queensland, 2016).

The leaf blades of diploid cultivars are generally translucent, bright green and narrower than the leaf blades of polyploids, which are coarser, thicker and blue-green in colour (Genovesi et al., 2009). The stolon internodes of diploid types are generally green, while stolon internodes of polyploids are generally purple (Busey, 2003b).

3.2 Reproductive morphology

Flowering stems (i.e. culms) also grow from the stolons of *S. secundatum*. These are short and upright and bear an inflorescence (Figure 1B) that is 20–150 mm long and 3–7 mm wide (Smith and Valenzuela, 2002; Busey, 2003b; Cook et al., 2005; Clayton et al., 2006; Biosecurity Queensland, 2016). The inflorescence is described as a modified spike-like panicle (Busey, 2003b), and the stem (i.e. rachis[9]) is a broad, flattened, wavy stalk (Sauer, 1972; Cook et al., 2005; Biosecurity Queensland, 2016), which is cork-like (Busey, 2003b). The inflorescences are mostly terminal but some may be auxiliary (Sauer, 1972; Duble (2001) cited in Hanna et al., 2013).

Individual spikelets or short clusters of spikelets (i.e. racemes[10]) are inserted alternately into the hollows on either side or on the face of the corky rachis (Busey, 2003b; Clayton et al., 2006; Biosecurity Queensland, 2016). The branches (i.e. pedicels) of the inflorescence bearing the spikelets are 2–4 mm in length (Biosecurity Queensland, 2016).

Spikelets are 3–6 mm long, hairless and awnless (Busey, 2003b). Each spikelet is made up of a pair of floral bracts, one of which is much smaller than the other, and two tiny florets (Figure 1C). The florets have a pair of bracts (i.e. a lemma and palea), the lower being sterile or having only male flower parts (i.e. three stamens) while the upper also has an ovary and a feathery two-branched stigma (Cook et al., 2005; Clayton et al., 2006; Biosecurity Queensland, 2016). In diploid types, the stigmata are usually whitish with yellow anthers, while in polyploids the stigmata are purple with orange-buff anthers (Busey, 2003b).

The 'seeds' (i.e. grains or caryopses) are dark brown, egg-shaped (i.e. ovoid), and about 1.5–2.0 mm long and 1.25 mm wide. They are shed from the seed head still contained within the old flower spikelets (Sauer, 1972; Cook et al., 2005; Biosecurity Queensland, 2016).

The descriptions provided in this section cover the variation across the species, however different types of *S. secundatum* characteristically will be smaller or larger than the norm, based on the demes described by Sauer (1972).

SECTION 4 PLANT DEVELOPMENT

4.1 Asexual reproduction

S. secundatum primarily reproduces asexually by the growth of rapidly spreading stolons (Busey, 2003b; CABI, 2014), rather than producing seed (Sauer, 1972). The stolon arising from a parent plant bears nodes from which self-sustaining plants can develop even if the physical connection with the parent is broken (Crampton, 1974). Sterile types of *S. secundatum*, particularly the common Cape

[9] A rachis is the stem or stalk that bears spikelets
[10] A raceme is a simple, unbranched inflorescence with stalked flowers.

deme, have spread and naturalised throughout the world due to the plant's ability to grow and reproduce asexually (Sauer, 1972; Busey, 2003b; Loch et al., 2009).

Asexual reproduction is enhanced in cultivated turf, where frequent mowing prevents seed set (Busey, 2003b), and rapidly spreading cultivars are continually selected (Loch et al., 2009).

4.2 Sexual reproduction

At the time of compiling this document, there was little literature regarding flowering, pollination, seed development and seed production for *S. secundatum*, reflecting that the species' primary means of reproduction is asexual and commercial development of cultivars is by vegetative propagation.

The potential for *S. secundatum* to reproduce sexually varies with ploidy levels and genotypes. However, even when natural conditions or cultivation practices facilitate seed production, spikelets often fail to mature (Sauer, 1972; Duble (2001) cited in Hanna et al., 2013) and seed yield is low (Busey, 2003b).

The foundation cultivar of commercial *S. secundatum* sod in the USA, Bitterblue, has a Cape deme genotype, and while it is a sterile clone, there is slight genetic variation within the cultivar (Busey, 1986). While triploid types are frequently referred to as sterile, some authors infer in their writing that, on occasion, these types may successfully flower and set seed (Busey, 2003b; Cook et al., 2005; Beard, 2012 and references therein). Cook et al. (2005) summarised that diploids are fertile, triploid types produce a little seed and tetraploid types are completely sterile.

Weedy and adventitious populations of the diploid Breviflorus Race, which are generally genotypes originating in the Gulf of Mexico region, have high seed set (Busey, 2003b). New 'soft-leaf' cultivars developed in Australia have their origin from selections of normal, fertile, diploid buffalo grass, naturalised along the mid-north coast of New South Wales. The first of these selections has been observed to set a high percentage of fertile seeds that readily germinate, although data supporting this assertion has not been published (Loch et al., 2009).

Within cultivated stands of buffalo grass, inbreeding depression is likely as seed has been produced from a clonal monoculture, and is therefore inbred (Busey, 2003b).

4.3 Pollination and pollen dispersal

When allowed to flower (i.e. not mown or grazed on a regular basis), *S. secundatum* can produce pollen over a period of up to four months in South Africa (Prescott and Potter, 2007).

S. secundatum pollen is comprised of 18 x 16 micron elongated spheres (Morgado et al., 2015). The pollen has no surface features suggesting adaptation to insect-mediated pollination, and the pollen is structurally similar to that of other grasses known to be wind-pollinated (Morgado et al., 2015). Furthermore, characteristic features of insect pollinated plants are absent from the reproductive structures of *S.secundatum,* whereas the plant possesses many of the features characteristic of wind pollinated plants. These include flowers exposed outside the leaves, petals small or absent, attractants absent, anthers and stigmas exposed, pollen grains small, smooth and dry, and high pollen production (Faegri and van der Pijl, 1979). One feature of *S. secundatum* that is not typically found in outcrossing wind-pollinated plants is that the upper floret is bisexual (Sauer, 1972). This may facilitate self-pollination.

As part of a population improvement program, Busey (2003b) successfully conducted hybridisation and self-crossing of *S. secundatum* with variable results in terms of the numbers and phenotypes of progeny. Busey (2003b) also reported that open pollination was achieved in greenhouse conditions, between two popular cultivars of different ploidy level, USA cultivar Floratam (2*n* = c.32) and polylpoid African parents (2*n* = 30), yielding genetically variable progeny.

4.4 Seed development and seed dispersal

Sauer (1972) concluded that perennial species of the *Stenotaphrum* genus, regardless of being sterile or fertile, produced little seed and propagated themselves vegetatively with great vigour. The seed of some genotypes of *S. secundatum* is viable as a result of cross pollination; other genotypes may self-pollinate but the seed is sterile (Beard, 2012). Triploids and tetraploid genotypes both form seeds that are sterile (Long and Bashaw, 1961), whereas, diploid genotypes form seeds with reasonable germination capability and considerable heterozygosity[11] (Beard, 2012).

Broadly, *S. secundatum* is reported to flower in the subtropics in the southern hemisphere from October to May but considerable variation in flowering time and intensity is noted between cultivars (Cook et al., 2005). The species is day-length sensitive and inflorescence initiation can be triggered by extending photoperiod (Genovesi et al., 2009). Studies in the USA indicate that there may be an interaction between long-day flowering response and temperature to initiate reproduction (Duff et al., 2009), and that photoperiod length and temperature were interacting inducers of flowering which tended to occur in June (Florida, USA) under day lengths of 13.5 hours (Dudeck, 1974); the same author also showed considerable intra-species diversity in this effect.

There has been no Australian research to determine the environmental requirements for buffalo grass to initiate its reproductive phase. Trials in Australia, of Australian and American commercial varieties, reported the production of seed heads during November (Duff et al., 2009). Seed head production continued for both groups through until February. Seed head production was higher, and started earlier, for the Australian Commercial group compared with the American Commercial group throughout the monitoring period. Variation in seed head production was also observed between cultivars (Duff et al., 2009).

Commercial (Buffalo Lawn Care) and scientific (Duff et al., 2009) literature indicates that seed heads readily develop in spring and summer when buffalo grass is not mown. Several other authors describe the appearance of the flowering parts of sterile and fertile genotypes, including Sauer (1972) and Busey (2003b). However, there is no literature describing the stages of development or the length of time from initiation of the seed head to seed maturation.

There is limited literature regarding seed dispersal for *S. secundatum.* The rachis (inflorescence) generally breaks at the branch nodes into squarish segments to which one or several spikelets are attached (Busey, 2003b). The seed is contained in a caryopsis, which is very difficult to remove from the rachis segments (Busey, 2003b). This structure has been attributed to the species' historical

[11] An individual having two different alleles for a trait, one from each parent.

distribution. The rachis segment enhances flotation on ocean currents between islands and along shorelines, and such segments can float in salt water for up to 10 days (Sauer, 1972; Beard, 2012).

4.5 Seed dormancy and germination

At the time of compiling this document, quantitative data regarding seed dormancy and germination rates was limited. Commercial product information (Buffalo Grass Review Site, Buffalo Lawn Care) describes germination as being poor and slow, and recommends against establishing buffalo lawn grass from seed.

4.6 Vegetative growth

S. secundatum is a perennial, warm-season, C4 plant that grows year round in subtropical and tropical environments but becomes dormant over winter in temperate environments (Aldous et al., 2014). It grows laterally by spreading, branching stolons (Busey, 2003b). Leaves establish at the nodes along the shoots, and lower leaves may be at right angles to the stem. Roots also develop at the nodes.

Diploid cultivars grow and cover an area more rapidly than polyploids due to diploids producing more branches (Busey, 1995). Trials of 17 Australian and American cultivars recorded plant diameters ranging between 70 cm and 200 cm 154 days after planting (Duff et al., 2009). Ploidy levels were not identified for the cultivars assessed in this trial work.

SECTION 5 BIOCHEMISTRY

5.1 Toxins

S. secundatum contains oxalic acid. However, the oxalic acid content of *S. secundatum* is approximately 1.2% of dry matter and this low concentration is not considered dangerous for livestock (Garcia-Rivera and Morris, 1955; Rahman et al., 2013). Oxalic acid has been described as potentially detrimental to the health of cattle at concentrations higher than 1.6% dry matter, due to disturbance of the metabolism of calcium and phosphorus (Garcia-Rivera and Morris, 1955; Mayland and Cheeke, 1995).

The grazing of *S. secundatum* by ruminants has been linked to Manchester wasting disease, a chronic condition characterised by weight loss and calcification of soft tissues (Arnold and Fincham, 1997). Manchester wasting disease is not perfectly associated with *S. secundatum* grazing, and occurs primarily in Jamaica where local nutrient or vitamin concentrations might lead to the disease. The disease resembles vitamin D toxicosis, and is most evident when vitamin D and calcium are in excess, and other nutrients are limiting - a condition found in buffalo grass monocultures in Jamaica (Arnold, 1969; Arnold and Fincham, 1997).

S. secundatum is not consumed by people.

5.2 Allergens

Pollen derived from *S. secundatum* has been described as a common sensitiser of grass-allergic subjects in South Africa (Prescott and Potter, 2007; Berman, 2011). People allergic to the pollen of

S. secundatum are also generally allergic to pollen of other subtropical grasses from the families Panicoideae and Chloridoideae (Prescott and Potter, 2007; Davies, 2014).

S. secundatum pollen is not considered a common allergen in Australia (Australasian Society of Clinical Immunology and Allergy) and is not reported as a clinically important source of subtropical grass pollen allergen (Davies, 2014). In the USA, pollen of *S. secundatum* is not commonly cited as a significant source of allergy (Pollen Library).

Compared with allergenic pollens derived from broadacre crops and pastures, and widespread major weeds in Australia, the volume of pollen originating from buffalo grass would be considerably less, due to the fact that most stands of buffalo grass are mowed regularly, preventing the development of flowers and subsequent pollen.

5.3 Beneficial phytochemicals

The nutritive value of *S. secundatum* as pasture has been described as being low (Macfarlane and Shelton, 1986; Smith and Valenzuela, 2002) and while suitable for maintenance nutrition, it may not be adequate for fattening stock (Macfarlane & Shelton 1986). Crude protein concentration of *S. secundatum* is generally lower than the 15% required for lactation and growth in grazing animals (Mullen and Shelton, 1996). Nutrient analysis shows that mineral concentrations in *S. secundatum* (Table 2) are not limiting factors for animal production (Mullen and Shelton, 1996).

Table 2. Mean mineral concentrations in plucked tips of *S. secundatum*, averaged over all soil type samples, compared with minimum levels for dietary intake. Adapted from Macfarlane & Shelton (1986)

	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sulphur	Sodium
% Dry matter	2.11	0.29	1.95	0.60	0.35	0.41	0.65
Min. % for growth	1.3-1.5	0.19	0.31–0.43	0.43	0.15	0.17	0.07
Min. % for lactation	—	0.23	—	0.32	0.18	0.17	0.10

Growing mixed pastures of *S. secundatum* and legume shrubs or climbers, such as *Leucaena leucocephala* or *Macroptilium atropurpureum*, is a recommendation to improve pasture quality (Macfarlane and Shelton, 1986; Smith and Valenzuela, 2002).

S. secundatum is a sodium accumulator, with concentrations in the shoots reaching up to 100 mM (Marcum and Murdoch, 1994; Heuzé et al., 2015). Salt supplementation is commonly recommended for cattle grazing tropical pastures however it is deemed unnecessary for cattle grazing *S. secundatum* pastures (Mullen and Shelton, 1996).

SECTION 6 ABIOTIC INTERACTIONS

S. secundatum is adapted to a wide range of environments due to the genetic and morphological diversity in the species, combined with selection and development of a wide range of cultivars for tropical, subtropical and temperate climates (Busey, 1995). Despite this diversity, there are some generalised responses to abiotic and biotic factors.

6.1 Abiotic stresses

6.1.1 Nutrient stress

The natural diversity of *S. secundatum* means that the species can be found growing on a range of soil types and in a range of conditions in natural environments. The species is primarily of tropical origin and naturally found on ridges of coastal sand dunes, the edges of swamps and lagoons, around salty and fresh water marshes and on limestone shorelines (Duble, 2005). Generally, *S. secundatum* is tolerant of low nutrient levels and will grow on low fertility soils (Smith and Valenzuela, 2002).

Under cultivation for turf, fertilisation practices are employed to maximise production and maintain soil fertility. Buffalo grass responds well to these increased nutrient levels (Smith and Valenzuela, 2002).

6.1.2 Temperature stress

Generally, *S. secundatum* grows best at temperatures between 20 °C and 30 °C, and the minimum temperature for growth is 10 °C (Cook et al., 2005). In temperate climate zones, the growth of buffalo grass slows in cooler months and the plant becomes dormant over winter. There is no quantitative data available regarding *S. secundatum* cold tolerance in Australia.

Winter survival of *S. secundatum* was determined in Florida after a severe cold front moved through the state in 1989, subjecting experimental plantings in 24 counties to a hard freeze and record low temperatures for several days (Busey, 2003c). With the exception of two counties, the limit for winter survival was a minimum air temperature between -6 °C and -9 °C. These observations correlate with the range of minimum temperatures at the northern limit of common occurrence of *S. secundatum* growing in unprotected climates in the south-eastern USA (Busey, 2003c).

The field results show greater winter survival than previous laboratory measurements of lethal temperatures for stolons and buds of *S. secundatum,* which were in the range of -4.5–7.7 °C (Busey, 2003c). There is ongoing work to develop better assessment methods of freeze tolerance between cultivars to extend the production and distribution of the species in the USA (Kimball et al., 2017).

In the USA, genotypic differences have been reported for winter survival, with cultivars derived from the sterile Cape deme being less cold tolerant than fertile diploid cultivars (Busey, 2003b, a). In Australia, commercial information about buffalo grass indicates that some cultivars are more tolerant to cold and frost than others (Buffalo Grass Review).

There is no literature available suggesting that buffalo grass will be adversely impacted by high temperatures. Management practices suggest that so long as water supply is adequate, the plant can endure high temperatures (see Section 6.1.3).

6.1.3 Water stress

Buffalo grass is adaptable to varying levels of soil moisture, from moderate water deficit to temporary flooding and waterlogging. It commonly grows in areas of 1000–2000 mm annual rainfall. However, if adequate soil moisture can be maintained, it will colonise areas of annual rainfall down to 750 mm (Cook et al., 2005).

Drought tolerance and drought recovery is of particular interest in Australia and overseas, as public and private water users strive for greater efficiency (Duff et al., 2009; Loch et al., 2009; Steinke et al., 2010; Colmer, 2012; Zhou et al., 2012).

S. secundatum has greater tolerance of water deficit than most other warm season turf grasses such as *Cynodon* spp. or *Zoysia* spp. because it has a deeper or more effective root system (Busey, 2003b). Polyploids show greater drought survival than diploids, with variation of drought survival between cultivars (Busey, 2003b).

The impact of soil depth on drought tolerance and drought recovery was investigated in Texas, USA. Cultivars of *S. secundatum* grown under restricted soil depth conditions (10 cm) survived drought stress for only 10 to 20 days, compared with plants grown under unrestricted soil depth conditions that survived drought stress for 60 days (Steinke et al., 2010). This work showed variation between cultivars, and consistently better drought tolerance and recovery by the sterile cultivar 'Floratam' compared with a range of other cultivars commonly used in Texas. Another study (Busey, 1996) demonstrated less leaf wilt under drought stress in polyploid lines compared with diploid lines.

When four warm-season turf grass species were compared, buffalo grass was generally ranked second for water use efficiency and survival under a range of irrigated and drought conditions (Zhou et al., 2012). New cultivars of buffalo grass had similar water use requirements to the common buffalo grass and showed similar recovery after periods of low water availability (Duff et al., 2009).

There is little information, technical or commercial, discussing the effect of waterlogging or flooding on buffalo grass. Draft Australian standards for use of turf for erosion control identify that buffalo grass can withstand temporary waterlogging. Contrasting reports suggest that *S. secundatum* is either moderately tolerant of waterlogging (Rogers et al., 2006), or does not tolerate waterlogging (Duble, 2005).

6.1.4 Other stresses

Soil pH

S. secundatum tolerates a wide range of soil pH. It grows successfully on soils ranging in pH from 4.5 to 8.5 but optimum soil pH for cultivation is between 5.0 and 7.0 (Smith and Valenzuela, 2002; Busey, 2003b; Duble, 2005; Aldous et al., 2014). In highly alkaline soils (above pH 7.5), leaves may become chlorotic (Duble, 2005).

Soil compaction

There is little information, technical or commercial, discussing the effect of soil compaction on buffalo grass. Agronomic information from the USA states that *S. secundatum* does not tolerate compacted soil conditions (Duble, 2005). The origin of the species as a coastal pioneer (Sauer, 1972) and general descriptions of the species' preference for lighter-textured soils (Busey, 2003b; Cook et al., 2005), suggests that the growth of *S. secundatum* would be negatively affected by compacted soils.

6.2 Abiotic tolerances

6.2.1 Shade

Globally, *S. secundatum* is recognised and described as one of the most shade-tolerant warm season turf grasses (White and Busey, 1987; Cook et al., 2005; Trenholm and Nagata, 2005; Aldous et al., 2014). Across much of Australia, buffalo grass is the most widely used lawn grass species for shaded areas (Duff et al., 2009).

Several studies report that *S. secundatum* grows better under 20 to 40 % relative irradiance than under 100% irradiance (full sunlight) (Shakesby, 1993; Smith and Valenzuela, 2002; Busey, 2003b). *S. secundatum* grown in pots was shown to produce 59% more dry matter under 32% relative irradiance than in full sun (Mullen and Shelton, 1996). The ability of *S. secundatum* to compete effectively with weeds under shade suits it for use as lawn in shaded gardens and for providing forage for livestock in plantations (Macfarlane and Shelton, 1986; Busey, 2003b).

Shade tolerance has been reported to vary between cultivars in the USA (Shakesby, 1993; Busey, 2003b; Trenholm and Nagata, 2005) and in Australia (Shakesby, 1993; Duff et al., 2009). In general, soft-leaf buffalo grass cultivars maintained an acceptable quality in a pot experiment in Queensland, where the shade level was below 70% (Duff et al., 2009).

6.2.2 Salt

Generally, *S. secundatum* is recognised as a moderately salt-tolerant grass for residential lawns, public spaces, and pastures (Dudeck et al., 1993; Cook et al., 2005; Aldous et al., 2014). The species also has potential to be grown on sites where saline or brackish water or effluents may be disposed of (Marcum and Murdoch, 1994), and/or there is increasing soil salinity (Loch et al., 2006). In Australia, buffalo grass has become naturalised in salt-affected areas near seawater (Loch et al., 2006).

Studies from the USA describe *S. secundatum* as a salt-tolerant warm season turf grass, with shoot and root growth being reduced by 50% at salinity of 32–36 dSm^{-1} and 44 dSm^{-1}, respectively (Marcum and Murdoch, 1994; Uddin et al., 2011). Turf quality was impacted at a salinity level close to seawater, 54 dSm^{-1} (Uddin et al., 2011). An Australian study at a salt-affected coastal site found that the salt tolerance of *S. secundatum* varied between cultivars, with a 50% reduction of dry matter at salinity of 9–21 dSm^{-1} (Loch et al., 2006).

6.2.3 Herbicide tolerance

S. secundatum has varying tolerances of different herbicides used in agricultural and horticultural production. There are a range of active ingredients that are registered as safe for use on buffalo grass (Table 3). A widely used product for the control of broadleaf weeds in buffalo grass is a mixture of bromoxynil and MCPA (Layt, 2017) but there is some variation in tolerance between cultivars to this product.

Buffalo grass is not tolerant or has low tolerance of some herbicides, such as dicamba, used for weed control in other lawn or turf grass species (Brosnan and DeFrank, 2008). Duff et al (2009) found dicamba to have no significant phytotoxic effect on any buffalo grass cultivar, however currently registered dicamba products stipulate that the product is not for use on buffalo grass (APVMA, 2017) and commercial information sites strongly advise against use of dicamba on buffalo grass (Layt, 2017).

Significant variation in tolerance of certain herbicides has been observed between cultivars (Duff et al., 2009). Some cultivars, e.g. the ST series[12], showed greater susceptibility than others to several herbicides. Generally, the cultivars evaluated by Duff et al (2009) showed good tolerance to the sulfonylurea group of herbicides (Group B). Some products, e.g. foramsulfuron (Group B), showed phytotoxic symptoms but 100% recovery was achieved in 14–28 days. Fluroxypyr (Group I) and chlorsulfuron (Group B) did not affect buffalo grass and were identified as potentially useful options for weed control and future consideration of registration. As at 2017, these products had not been registered for use with buffalo grass (APVMA, 2017).

Tolerance (or sensitivity) to herbicides may vary with the growth stage of the plant. Juvenile turf is very sensitive to herbicide damage and herbicides should not be applied during the first three to four months of establishment (Aldous et al., 2014).

Table 3. Selective herbicides registered by APVMA for weed control in *S. secundatum* in Australia. Database accessed 19 July 2017.

Active ingredient	**Herbicide group**	**Notes*** APVMA - PubCris
Bromoxynil	C	Post-emergent control for broadleaf weeds; often mixed with MCPA
Carfentrazone-ethyl	G	
Diclofop-methyl	A	Post-emergent control of grass species, crowsfoot
Disodium methylarsonate (DSMA)	Z	
Dithiopyr	D	Pre-emergent weed control of certain summer grass, annual grasses and broadleaf weeds; post-emergent control of summer grass
Endothal	Z	
Halosulfuron-methyl	B	
Indaziflam	O	
Iodosulfuron-methyl-sodium	B	Post-emergent control of certain broadleaf weeds and grasses
Iron as ferrous sulfate	—	
Isoxaben | florasulam	N	
MCPA	I	
Metolachlor	K	
Monosodium methylarsonate (MSMA)	Z	
Oryzalin	D	
Oxadiazon	G	
Pendimethalin	D	

[12] Buffalo grass varieties developed in the USA but propagated and produced for sale in Australia.

Prodiamine	D	Pre-emergent control for grass weeds
Propyzamide	D	

* information sourced from Public Chemical Registration Information System Search (APVMA)

SECTION 7 BIOTIC INTERACTIONS

7.1 Weeds

A productive, well-maintained sward of buffalo grass will suppress weed growth, and this feature makes buffalo grass a sought-after lawn species. The tight leaf canopy and relatively prostrate leaf angle are key attributes that make *S. secundatum* lawns highly resistant to weed infestation (Busey, 2003b). Frequent mowing of cultivated lawns also reduces the opportunity and pressure of weed infestations, although mowing height is important, to ensure developing weed seed heads are removed (Busey, 2003a; Duff et al., 2009; Aldous et al., 2014).

Weed infestation is likely when the health of the sward is compromised, and ground cover becomes patchy due to insufficient water, excessive wear and traffic, poorly managed nutrition, insect and disease infestation or cold damage (Busey, 2003a; Aldous et al., 2014). Weed management is discussed in Section 2.3.3.

Typical broadleaf weeds of buffalo grass in Australia include bindii (*Soliva pterosperma*), clover (*Trifolium* spp.), cats ear (*Hypochaeris radicata*) and dandelion (*Taraxacum officinale*). Grass weeds include paspalum (*Paspalum* spp.), winter grass (*Poa annua*), kikuyu (*Pennisetum clandestinum*) and couch grass (*Cynodon dactylon*) (Buffalo Lawn Care).

7.2 Pests and pathogens

A healthy and vigorously growing sward of buffalo grass, that is well-maintained and well-managed, will generally be able to withstand incursions of small insect populations, and recover from damage or injury caused by insects and disease (Aldous et al., 2014). However, a pest or disease outbreak may be facilitated by certain environmental conditions, particularly temperature and humidity (Layt, 2017), or poor management in regards to fertilisation, irrigation and mowing (Aldous et al., 2014). The development of a thick thatch, due to lack of renovation, provides harbour for disease pathogens and pest larvae (Colmer, 2012; Aldous et al., 2014). Pest and disease management is discussed in Section 2.3.3.

Turf grass species may be infested with a range of chewing and sucking pests, above and below the soil surface (Aldous et al., 2014). Potential pests include beetles, weevils, mole crickets, web worm, army worm, cut worm, nematodes, grasshoppers, leaf bugs, ants, centipedes and millipedes, aphids, leafhoppers, scale insects, and spider and clover mites (Aldous et al., 2014). The most common pests in buffalo grass are web worm, army worm and African black beetle, in the warmer months of the growing season (Layt, 2017).

In Australia, pathogens causing disease in buffalo grass may include *Rhizoctonia* spp. (brown patch), *Pyricularia grisea* (grey leaf spot), *Sclerotinia homeocarpa* (dollar spot) and a range of fungal species causing powdery mildew (Buffalo Lawn Care). These pathogens also may affect other grass species, and some fungi may affect other non-grass plant species.

SECTION 8 WEEDINESS

In Australia, buffalo grass is important commercially as a turf grass species, and deliberately cultivated in home gardens and public open spaces. Naturalised buffalo grass is identified as an environmental weed in natural environments (Muyt, 2001).

Buffalo grass (developed or naturalised) has some key characteristics that led to its initial introduction across the world and then its development and improvement as a turf species. *S. secundatum* is a perennial, shade-tolerant grass, with the potential to produce tight, dense canopies. Dry matter production has been reported to be 10–20 tonnes per hectare per year (Mullen and Shelton, 1996). These characteristics can also contribute to weediness.

S. secundatum lacks the ability to produce high numbers of viable seed, which is a characteristic common to many weeds; therefore weediness of *S. secundatum* linked to seed bank persistence is limited (see Section 4.4). However, the species reproduces vegetatively and is regarded as an invasive weed in natural environments due to its potential to form a dense mat that can eliminate other ground flora and prevent regeneration of overstorey species (Muyt, 2001). *S. secundatum* can usually be eliminated over a single growing season by herbicide treatment (Muyt, 2001).

8.1 Weediness status on a global scale

An important element in predicting weediness is a plant's history of weediness in any part of the world (Panetta, 1993; Pheloung, 2001).

S. secundatum is not considered a weed in the USA (USDA Plant Database; accessed on 31 October 2017). However, it is listed as "likely to be invasive in Hawaii and on other Pacific Islands" by Daehler et al. (2004). These authors describe *S. secundatum* as an agricultural and environmental weed, due to its shade tolerance, ability to grow on a wide range of soil conditions and its capacity to reproduce by vegetative fragmentation. *S. secundatum* is recorded as an invasive species in a national park in Hawaii (Invasive Plant Atlas of the United States).

S. secundatum is considered an environmental weed in New Zealand, in disturbed shrub lands and coastal fringes (Weedbusters; accessed on 22 June 2016). Further, *S. secundatum* is potentially able to displace rare or threatened species in coastal areas of Spain and is categorised as a transformer[13] (Campos et al., 2004).

S. secundatum is characterised by its rapid, vigorous growth (Shelton, 1991). For example, in Sulawesi, *S. secundatum* cuttings planted at 1 m x 1 m spacings fully covered one hectare after four months of growth (Mullen and Shelton, 1996 and references therein). Another characteristic of the species is its ability to maintain yield at low light transmission, giving it a competitive advantage in shaded environments (Mullen and Shelton, 1996). While these characteristics of *S. secundatum* may dispose the species to weediness, Song et al (2013b) found that the regenerative capacity of several stoloniferous clonal grasses showed no relationship to the actual invasiveness of a species in China or globally (Song et al., 2013b). Although this study did not include *S. secundatum*, it did include *S. dimidiatum* and other similar grasses such as *Cynodon dactylon* (couch grass) and *Zoysia japonica* (zoysia grass).

[13] An invasive plant that changes the character of an ecosystem.

8.2 Weediness status in Australia

S. secundatum is not classified as a noxious weed in any state or territory in Australia, and is not included in any federal government weeds list (National Weeds Lists).

In the context of weed science *S. secundatum* has been identified as a weed of the natural environment, a weed escaped from cultivation and a weed of agriculture in Australia (Randall, 2007). While the weed has been designated as an invasive species overseas, indicating that it can be a high impact weed that spreads rapidly and may create monocultures, it is not considered an invasive species in Australia (Randall, 2007). A global weed risk score for *S. secundatum* was calculated as 35.84 on a scale from 0-64, which places it in the top 3% of assessed plants in terms of weediness (Randall, 2016, 2017).

The weediness of introduced flora in Australia was also categorised by Groves et al (2003). On a scale of 0-5, *S. secundatum* was classified as a category 3 weed in natural ecosystems, indicating that it was known to be a minor problem warranting control at four or more locations within a state or territory. Using the same system, *S. secundatum* was classified as category 1 weed in agricultural ecosystems, Australia-wide, indicating that it may be a minor problem but was not considered important enough to warrant control.

S. secundatum is regarded as an environmental weed in Victoria, Western Australia, New South Wales, South Australia and south-eastern Queensland (Biosecurity Queensland, 2016).

A weed risk assessment for *S. secundatum* based on the Australian/New Zealand National Post-Border Weed Risk Management Protocol is found in Appendix A.

8.3 Weediness in agricultural systems

The identified potential for *S. secundatum* to be a weed of agricultural systems (Randall, 2007) is based on some key characteristics and attributes of the species such as:

- the ability to reproduce by vegetative means from inadvertent dispersal of fragments of plants (Sauer, 1972)
- its adaptability to a wide range of environments, from temperate to tropical environments and across a wide range of soil types (Sauer, 1972)

At the same time, some key characteristics and attributes of the species limit its invasive and competitive ability in agricultural systems. These features include:

- slow rate of establishment from sprigs or seed (Smith and Valenzuela, 2002) therefore its likeliness to be out-competed when spreading from propagules
- better growth in shaded sites (Smith and Valenzuela, 2002) compared with the open and well-lit situation of broadacre agriculture and horticulture, with the exception of large tree plantations (Mullen and Shelton, 1996)
- its low seed set (Sauer, 1972).

8.4 Weediness in natural systems

In Australia, naturalised *S. secundatum* is considered most troublesome in light, sandy soils in coastal districts (Muyt, 2001). In coastal areas it is observed to replace native grasses and invade native areas of shrubs and trees due to its tolerance of shaded environments (D. Loch, personal communication, 2018) (Figure 2).

Figure 2. Invasive *S. secundatum* on NSW south coast. D. Loch, 2017.

Impact studies of *S. secundatum* have been conducted in different natural environments within Australia. *S. secundatum* has been described as non-invasive in Royal National Park, NSW (Murray and Phillips, 2010). However, it has been identified as having a negative impact on two threatened species on Lord Howe Island (Coutts-Smith and Downey, 2006). For one of these species, *Caesalpinia bonduc* (grey nick), this is due to the impact of *S. secundatum*'s very dense cover on seed recruitment. For the other species, *Calystegia affinis* (Lord Howe Island morning glory), negative impact is linked to competition with *S. secundatum*.

Other studies have highlighted the potential risk posed by *S. secundatum* to native communities across wetlands and coastal regions of Australia (Davis and Froend, 1999; Gooden and French, 2014). Exotic grasses, including *S. secundatum*, are described as able to smother the native understorey species, increase the fire risk and limit the establishment of tree seedlings (Davis and Froend, 1999). Impact on native plant communities described in Gooden & French (2014) was not clear. Similarly, *S. secundatum* was identified as the dominant species for one of seven plant communities in grassy coastal headlands of northern NSW (Hunter and Hunter, 2017) but the report did not discuss its impact on the natural ecosystem.

The presence of *S. secundatum* in natural ecosystems appears to be linked to disturbance. *S. secundatum* is found on Lady Elliott Island, QLD but not on any other island of the Great Barrier

Reef, even though *S. secundatum*'s seeds can be locally dispersed by ocean currents (Mullen and Shelton, 1996; Batianoff et al., 2009). Lady Elliott Island has the highest level of disturbance compared with the other islands in the area (Batianoff et al., 2009).

The spread of buffalo grass as a weed is an existing problem and some local and state government authorities provide educational material to the public in an attempt to reduce its impact on natural environments (Brisbane City Council, 2016; Western Australian Herbarium, 2017). Spread by stolons is considered more likely than dispersal *via* seed. Stolons may be spread in garden waste and during sod transportation, as well as spread by water, animals and vehicles (Brisbane City Council, 2016).

8.5 Control measures

The most effective control of buffalo grass is by chemical means, with the application of herbicides. Buffalo grass may be controlled with glyphosate (CABI, 2014; HerbiGuide, 2014; APVMA, 2017), applied as a blanket spray to an area or by painting runners or crowns where fragments exist, small plants exist amongst desired plants, or the buffalo grass is in a sensitive environment (HerbiGuide, 2014). There may also be selective herbicides available to control buffalo grass amongst broadleaved plants (HerbiGuide, 2014).

Repeated cultivation of an area may provide control or the thatch and topsoil could be physically removed (Buffalo Lawn Care). While physical control is possible, the ability of the species to readily propagate from plant fragments could make physical removal unreliable (HerbiGuide, 2014), as well as damaging to any nearby desirable plants.

Effective control requires the removal or killing of all runners, and regardless of physical or chemical approaches, follow-up manual removal of fragments may be required for complete control (HerbiGuide, 2014).

SECTION 9 POTENTIAL FOR VERTICAL GENE TRANSFER

Vertical gene transfer is the transfer of genetic material from parents to offspring, which occurs in sexual and asexual hereditary processes. Potentially, gene transfer can be intraspecific, interspecific or intergeneric. This section focuses on gene transfer by sexual reproduction. Asexual reproduction is discussed in Section 4.1.

Successful gene transfer requires three criteria to be satisfied. The plant populations must overlap spatially; they must overlap temporally (including flowering duration within a year and flowering time within a day) and the plants must be sufficiently close biologically to produce fertile hybrids, which will facilitate introgression into a new population (den Nijs et al., 2004).

9.1 Intraspecific crossing

Intraspecific gene transfer is the transfer of genetic material from parent to offspring by reproduction within the same species, through sexual or asexual reproduction. Intraspecific crossing is hybridisation between two plants of the same species.

Sexual reproduction is possible within fertile cultivars (Busey, 2003b), and is discussed in more detail in Section 4 *Plant development*. However, given the vegetative growth habit and perennial nature of *S. secundatum*, any intraspecific crossing that occurs is likely to be between genetically similar parents. Plants arising from successful crossing generally have low seed yield, seed germination levels are low and inbreeding depression is likely (Busey, 2003b). Crossing between different ploidy levels is rarely successful.

There is no quantitative information regarding intraspecific gene transfer of *S. secundatum* in Australia, however the 'soft-leaf' buffalo grass cultivar, Shademaster, sets a high percentage of fertile seed, which germinates readily (Loch et al., 2009). Australian turf industry members claim that commercially successful diploid cultivars Palmetto and Sapphire normally produce little to no seed (Buffalo Lawn Care), but little scientifically published data are available to support this claim.

9.2 Natural interspecific and intergeneric crossing

Interspecific and intergeneric crossing is the hybridisation between plants of different species but the same genus, and between plants from different genera, respectively.

Apart from *S. secundatum*, the only other species within the *Stenotaphrum* genus that has been described in Australia is *S. micranthum* (Atlas of Living Australia). No natural hybridisation events have been reported between *S. secundatum* and *S. micranthum*, in Australia or elsewhere. Based on morphological observations, some polyploid *S. secundatum* cultivars developed with African germplasm show possible introgression with *S. dimidiatum* (pemba grass), which is used as a lawn species in Africa and India (Sauer, 1972; Busey, 2003b). *S. dimidiatum* is native to islands of the Indian Ocean.

It was proposed by Sauer (1972) that the *Stenotaphrum* genus evolved from the *Paspilidium* genus, and that *S. secundatum* was third in a line of divergent species evolving from *Paspilidium,* after *S. helferi* and *S. dimidiatum.* However, this suggestion is not universally accepted (Webster, 1988). No published data can be found to suggest that natural crossing occurs between *S. secundatum* and species of the *Paspilidium* genus.

Estimation of genetic diversity and morphological relationships between turf grass species found *S. secundatum* (cultivar Raleigh, 2n=18) was most closely related to *Pasapalum notatum* (bahia grass) and *P. vaginatum* (seashore paspalum or saltwater couch). To a lesser extent it was related to *Pennisetum clandestinum* (kikuyu) (Budak et al., 2004). No published data can be found to suggest that natural crossing occurs between *S. secundatum* and these relatives.

9.3 Crossing under experimental conditions

There has been some deliberate crossing of *S. secundatum* under experimental conditions but the extent of this is limited, probably due to commercial plant breeding and cultivar development of *S. secundatum* largely being conducted by selection of naturally occurring variants and subsequent vegetative propagation (Busey, 2003b; Budak et al., 2004; Loch et al., 2009).

Busey (2003b) conducted a population improvement program, using parents from several different taxonomic groups within *S. secundatum* (Busey et al., 1982). Parent plants were randomly hybridised and self-crossed to produce offspring that achieved variable results in terms of numbers and phenotypes of progeny (Busey, 2003b).

Hybrids between *S. secundatum* varieties with different ploidy levels have been generated using embryo rescue technology (Genovesi et al., 2009), and the first commercial cultivar developed by this method was released in the USA in 2014 (Chandra et al., 2015).

In 2017, the first hybrids between *S. dimidiatum* (L.) Brongn (pemba grass) and *S. secundatum* were reported (Genovesi et al., 2017). This interspecific crossing aimed to introduce resistance to a number of insects, pathogens and nematodes to *S. secundatum*. Embryo rescue techniques were required to recover seedlings as seed from the crosses was small, shrivelled, lacking fully developed endosperm, and unable to germinate.

SECTION 10 SUMMARY

This document provides baseline information about buffalo grass (*Stenotaphrum secundatum* (Walter) Kuntze). The information included relates to the taxonomy and cultivation of *S. secundatum*, general descriptions of its morphology, reproductive biology, biochemistry, biotic and abiotic interactions, its weediness and the potential for gene transfer to occur to closely related species. The purpose of this baseline document is to inform risk assessments of genetically modified *S. secundatum* that may be released into the Australian environment.

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APPENDIX A WEED RISK ASSESSMENT OF BUFFALO GRASS

Species: *S. secundatum* (buffalo grass)

Relevant land uses[14]:

1. Conservation and natural environments (Class 1.1 – Nature conservation)
2. Production from irrigated agriculture and plantations (Class 4.5.4 — Irrigated turf farming)
3. Intensive uses (Class 5.5.3 — Recreation and culture)
4. Intensive uses (Class 5.4 — Residential and farm infrastructure)

Background: In Australia, buffalo grass occurs in a wide range of environments, as deliberate plantings and volunteer populations. Buffalo grass is cultivated on irrigated turf production farms where improved cultivars are grown to produce sod or ready-to-lay turf for home gardens, public gardens and public amenities, such as green spaces and roadsides. Historically, buffalo grass was introduced to Australia and transplanted for lawns, pasture and soil stabilisation. Buffalo grass becomes a weed when its range of growth extends beyond the boundaries of areas of deliberate plantings, which is facilitated by its stoloniferous growth habit. This weed risk assessment is for **non-GM buffalo grass volunteers**.

Weeds are usually characterised by one or more traits, such as rapid growth to flowering, high seed output, and tolerance of a range environmental conditions. Further, they may cause harm to human health, safety and/or the environment. Although buffalo grass has some traits associated with weeds, it is not a declared invasive weed in Australia. While the creeping, stoloniferous growth habit of buffalo grass means that the plant can easily and steadily escape areas of cultivation, it is not an aggressive competitor, and it does not produce high numbers of fertile seed. Groves et al. (2003) described *S. secundatum* as a naturalised non-native plant species[15] and classified it as a category 3[16] weed in natural ecosystems and category 1[17] weed in agricultural ecosystems, Australia-wide.

The Weed Risk Assessment (WRA) methodology is adapted from the Australian/New Zealand Standards HB 294:2006 National Post-Border Weed Risk Management Protocol. The questions and ratings used in this assessment are based on the South Australian Weed Risk Management Guide (Virtue, 2004). Questions 1–5 relate to the invasiveness of buffalo grass and questions 6-11 relate to the impact of buffalo grass on relevant land use area. Unless cited, information in this appendix is sourced from the main document, *The biology of* Stenotaphrum secundatum *(Walter) Kuntze – Buffalo grass.*

This risk assessment is consistent with previous assessments of *S. secundatum* in Australia described in Section 8.2 and provides a baseline for the assessment of GM *S. secundatum* crops.

[14] (ABARES, 2016) Version 8 October 2016

[15] A species that has been introduced, become established and that now reproduces naturally in the wild without human intervention (Groves et al., 2003)

[16] Naturalised and known to be a minor problem warranting control at 4 or more locations within a state or territory.

[17] Naturalised and may be a minor problem but not considered important enough to warrant control at any location.

1. Invasiveness of buffalo grass

Invasiveness questions	**Buffalo grass (*S. secundatum*)**
1. What is the ability of buffalo grass to establish amongst existing plants?	**Rating: Low – Medium** Primarily, buffalo grass establishes by the growth of stolons rather than seedlings. It is a ruderal species, and a poor competitor with existing plants. Historically, its spread in Australia has been in areas where there was little or no existing ground cover, e.g. sand dunes. Buffalo grass is likely to have a low–medium ability to establish amongst existing plants, with a greater ability to establish in more sparsely vegetated areas such as nature conservation land uses or poorly vegetated areas around residential, municipal and agricultural infrastructure land uses, than in more densely vegetated situations of agricultural and plantation land uses. The ability of buffalo grass to compete with existing plants is greater in shaded conditions.
2. What is the tolerance of buffalo grass to average weed management practices in the land use?	**Rating: Low – Medium** Buffalo grass is susceptible to several broad spectrum herbicides that are used for the control of a range of weeds in agricultural production areas, in residential gardens and in open urban and recreational spaces. In agricultural land uses, buffalo grass has low tolerance of primary cultural methods of weed control, in particular the establishment of and management for dense plant populations of the target crop or pasture. Buffalo grass would be relatively tolerant of land cultivation, which may be a weed control practice in many land uses. Cultivation may destroy a portion of established plants but in suitable conditions, fragments of stolons may be able to re-establish. In recreational, residential or roadside land uses, physical methods of weed control such as mowing or slashing will not control buffalo grass. There may be instances where buffalo grass is a 'welcome' volunteer in these land uses.
3. Reproductive ability of buffalo grass in the land use:	
3a. What is the time to seeding in the land uses?	**Rating: < 1 year** Although buffalo grass is perennial, it develops to maturity in one growing season. Thus, if the buffalo grass is a fertile cultivar,

Invasiveness questions	**Buffalo grass (*S. secundatum*)**
	seeds will be produced in less than one year.
3b. What is the annual seed production in the land use per square metre?	**Rating: Unknown** Buffalo grass develops seed heads when left unmown. However, for many cultivars and naturalised types, if seed set does occur, seeds are infertile. There is no peer-reviewed published information on volume of seed production in buffalo grass in Australia.
3c. Can buffalo grass reproduce vegetatively?	**Rating: Frequent** The primary means of reproduction of buffalo grass is by the development of stolons, and growth of new stems and roots from the nodes along the stolon. Vegetative growth and reproduction is the key means of plant development in both cultivated and uncultivated situations.
4. Long distance dispersal (more than 100 m) by natural means in land uses	
4a. Are viable plant parts dispersed by flying animals (birds and bats)?	**Rating: Unlikely** The dispersal of viable segments of buffalo grass by flying animals has not been reported in Australia or overseas. Further, the success of dispersal would depend on stolon segments being transferred to a conducive location to take root, which requires adequate moisture availability and soil contact.
4b. Are viable plant parts dispersed by wild land-based animals?	**Rating: Unlikely** The dispersal of viable plant parts is unlikely and there is no information to suggest this type of dispersal in Australia. While the plant may be foraged by wild land-based animals, including hooved animals, the opportunity for stems and stolons becoming attached to hooves or other body parts is limited as buffalo grass has no particular features such as burrs, barbs or sticky surfaces that would attach to fur or feet of animals. Further, the success of dispersal would depend on stolon segments being transferred to a conducive location to take root, which requires adequate moisture availability and soil contact.
4c. Are viable plant parts dispersed by water?	**Rating: Occasional** The most likely avenue of dispersal of viable plant parts by water is the transportation of stolon fragments from turf production, gardens or waste. Turf production areas generally are in close

Invasiveness questions	**Buffalo grass (*S. secundatum*)**
	proximity to waterways that supply and drain irrigation water. In nature conservation land uses, buffalo grass generally establishes in wet or frequently damp areas, which may become flooded regularly or on occasions, providing opportunities for viable segments of buffalo grass to be carried downstream or further away from the watercourse and subsequently establishing in suitable conditions. The morphology of buffalo grass facilitates short-term (7–10 days) transport of segments of the inflorescence in water. However, in cultivation, the development of seed heads is infrequent. The viability of any seed dispersing in this manner will depend on the parentage of the seed.
4d. Are viable parts dispersed by wind?	**Rating: Unlikely** Infrequent production of fertile seed reduces the opportunity for seed dispersal. Further, seed is contained in a caryopsis, which is very difficult to remove from the rachis segments, and seed heads develop close to the ground reducing the likelihood of wind dispersal. Stolon fragments that could form new plants are unlikely to dispersed by wind.
5. Long distance dispersal (more than 100 m) by human means in land uses	
5a. How likely is deliberate spread via people?	**Rating: Common** *S. secundatum* has been spread deliberately throughout the world by humans (as well as other natural means) as a species for lawns in gardens and public areas, ground cover and/or pastures in plantations, and pastures in subtropical and tropical areas. The selection and transplanting of runners or sprigs of buffalo grass from established lawns or other swards to establish new lawns occurs regularly. The species is deliberately spread by people through the cultivation of buffalo grass turf for installation on properties potentially up to several hundreds of kilometres away from the turf farm.
5b. How likely is accidental spread via people, machinery and vehicles?	**Rating: Occasional – common** Accidental dispersal of plant fragments could occur during the deliberate transfer of buffalo grass from one garden to another. The turf market involves transporting ‘live’ turf from the turf farm

Invasiveness questions	**Buffalo grass (*S. secundatum*)**
	to the customer, where there could be an opportunity for fragments to break off turf rolls and establish where they land. Accidental dispersal could be associated with the disposal of garden waste, or transfer of ground cover and topsoil from building sites. Small viable sprigs of the plant may have the opportunity to transfer to non-target areas on the tyres and wheels of vehicles and machinery. The success of dispersal would depend on sprigs being transferred to a conducive location to take root, which requires adequate moisture availability and soil contact. The plant fragments and occasional seed produced by buffalo grass are not reported to be particularly adhesive to clothing and vehicles. No specific structures mediating this mode of transport are evident on buffalo grass.
5c. How likely is spread via contaminated produce?	**Rating: Unlikely** As there is no seed production industry for buffalo grass in Australia, it is very unlikely that seed for other lawn or turf species would be contaminated with buffalo seed grass, or that agricultural or horticultural produce would be contaminated with buffalo grass seed. It is unlikely that most types of agricultural or horticultural produce would be contaminated with vegetative segments of buffalo grass, due to the rare occurrence of produce being harvested, handled or stored in an area where buffalo grass is growing. However, buffalo grass could feasibly be a contaminant of landscaping materials (soil, sand, gravel and mulch) given these materials are sourced and stored in areas conducive to the growth of buffalo grass. Buffalo grass may also contaminate the sod of another turf species, however quality control systems for turf production endeavour to maintain product integrity.
5d. How likely is spread via domestic/farm animals?	**Rating: Occasional** Buffalo grass is rarely used as a pasture species although there may be some volunteers in pastures on grazing properties, or in unmanaged areas of these farms, e.g. near infrastructure, along fence lines and laneways, and adjacent to waterways. Fragments of plants could be spread in mud on animal hooves as animals are moved from one paddock to another, to a feedlot or to other farms, but the likelihood of this occurring is limited as the buffalo

Invasiveness questions	**Buffalo grass (*S. secundatum*)**
	grass has no particular features such as burrs, barbs or sticky surfaces that would attach to fur or feet of animals. The success of dispersal would depend on stolon segments being transferred to a conducive location to take root, which requires adequate moisture availability and soil contact. There is no peer-reviewed information discussing the possibility of seeds passing through the digestive tract of animals undamaged, however this is a viable mode of transport in other grass species.

2. Impact of buffalo grass

Impact questions	**Buffalo grass (*S. secundatum*)**
6. Does buffalo grass reduce the establishment of desired plants?	**Rating: <10% reduction** Once established, buffalo grass forms a thick dense thatch that will reduce, and for some species, prevent, the establishment of other plants. There is limited research on the impact of buffalo grass in natural environments, but it has been established that buffalo grass thatch limits the recruitment of native species, but does not reduce seed bank richness (Gooden and French, 2014). In agricultural crops, buffalo grass has low tolerance of primary cultural methods of weed control, and has not been reported as limiting the establishment of desired plants. Similarly on turf farms, buffalo grass is susceptible to control measures and unlikely to affect the establishment of a turf of another species.
7. Does buffalo grass reduce the yield or amount of desired vegetation?	**Rating: < 10% reduction** In home and public gardens, runners of volunteer buffalo grass may encroach on bare soils and prevent the growth and coverage expected of desired plants. Buffalo grass is used as ground cover in plantations, suggesting that it does not reduce the yield of plants that have taller canopies and deeper root systems than buffalo grass.
8. Does buffalo grass reduce the quality of products or services obtained from the land use?	**Rating: No reduction–low** The establishment of volunteer buffalo grass could reduce the quality of products and services on some land uses. On land used for nature conservation, buffalo grass may marginally reduce biodiversity; in turf cultivation it may enter sod production areas

Impact questions	**Buffalo grass (*S. secundatum*)**
	of other turf species and reduce product quality; in pasture areas buffalo grass may be less nutritious than preferred pasture species.
9. What is the potential of buffalo grass to restrict the physical movement of people, animals, vehicles, machinery and/or water?	**Rating: None – low** Buffalo grass is a low growing plant and if established as a volunteer would not restrict the movement of people, animals, vehicles or machinery. Thatches that have had the opportunity to develop in channels or waterways may slow down the flow of water, however its low growth habit would not prevent flow. Buffalo grass is sensitive to prolonged waterlogging so would not survive in permanently or frequently inundated waterways.
10. What is the potential of buffalo grass to negatively affect the health of animals and/or people?	**Rating: None** While the pollen of *S. secundatum* is allergenic, it is not considered a clinically important subtropical grass pollen allergen source in Australia. Further, the volume of pollen originating from buffalo grass would be considerably less than pollen from other grasses and crops, due to regular mowing of buffalo grass, preventing the development of seed heads and pollen. While buffalo grass contains oxalic acid reports of adverse effects on livestock health are few. Almost exclusive consumption of *S. secundatum* was linked to Manchester wasting disease in livestock in Jamaica, due to high levels of vitamin D intake. The intake of forage from volunteer stands of buffalo grass is likely to be small, compared with intake of other pasture species, and therefore there is limited opportunity for negative effects on animal health.
11. Major positive and negative effects of buffalo grass on environmental health in the land use	
11a. Does buffalo grass provide food and/or shelter for pests and pathogens in the land use?	**Rating: Minor or no effect** Volunteer buffalo grass may lead to the formation of thick, dense thatch, which can harbour disease pathogens and insect larvae. The density of the thatch can make the control of pests and insects through application of pesticides difficult. In Australia, buffalo grass is susceptible to a range of diseases including rhizoctonia, grey leaf spot, dollar spot and powdery mildew (caused by a range of fungal species). These diseases may affect other grasses and some, e.g. powdery mildew, may affect other plant species. There is the potential for transfer of some

Impact questions	**Buffalo grass (*S. secundatum*)**
	pathogens from buffalo grass volunteers to cultivated buffalo grass or to other plants species. These pathogens also affect other grasses and plants, and their prevalence depends on seasonal conditions and a suitable host crop. Similarly, there is a range of pests that take shelter and feed in buffalo grass thatch, and these have potential to affect deliberate plantings of buffalo grass in residential, infrastructure and turf production land uses; as well as other plant species. Pest species that could be expected in buffalo grass stands include army worm, cutworm, black lawn beetle, ants and sod webworm. As with pathogens, there is potential for transfer of some pests from volunteer buffalo grass to cultivated buffalo grass or other species. These pest species also affect other grasses and plants, and their prevalence depends on seasonal conditions and a suitable host crop.
11b. Does buffalo grass change the fire regime in the land use?	**Rating: Minor or no effect** Volunteer buffalo grass will not increase fire risk in any of the land uses it may occur. Primarily its low growing habit and long growing season (keeping it green throughout summer and autumn) reduces fire risk compared with Mediterranean species in the same environment. In fact, buffalo grass is a recommended species for creating a "defendable area" around homes in bushfire-prone areas (Country Fire Authority).
11c. Does buffalo grass change the nutrient levels in the land use?	**Rating: Minor or no effect** As with all plants, buffalo grass will use soil nutrients for growth. While buffalo grass in cultivation responds well to fertilisation, unfertilised, its growth rate is much slower and nutrient depletion will not affect other species in the area or subsequent species planted in the same area.
11d. Does buffalo grass affect the degree of soil salinity in the land use?	**Rating: Minor or no effect** As a ruderal weed, volunteer buffalo grass may have a small positive effect on soil salinity, i.e. reducing the extent of percolation of water and salt transport through the soil profile by active transpiration in an otherwise non-vegetated area.
11e. Does buffalo grass affect the soil stability in the land use?	**Rating: Minor or no effect** As a volunteer weed, the presence of buffalo grass may have a positive effect in stabilising light-textured or sandy soils. In shaded

Impact questions	Buffalo grass (*S. secundatum*)
	areas, it may provide ground cover where other grasses struggle to survive.
11f. Does buffalo grass affect the soil water table in the land use	**Rating: Minor or no effect** As a ruderal weed, volunteer buffalo grass may have a small positive effect on the soil water table, i.e. by reducing the extent of percolation of water through the soil profile by active transpiration in an otherwise non-vegetated area.
11g. Does buffalo grass alter the structure of nature conservation by adding a new strata level?	**Rating: Minor or no effect** Published literature on buffalo grass impact on nature conservation land uses is restricted to one study of 500 km of coastline in south eastern Australia (Gooden & French 2014). The morphology of the plant is similar to other grasses that are widespread in Australia and therefore buffalo grass would not add a new strata level to nature conservation.