

Research Paper

Selection for resistance to fungal diseases and other desirable traits in kikuyu grass (*Cenchrus clandestinus*)

Selección por resistencia a enfermedades fungosas y otras características deseables en germoplasma del pasto kikuyo (Cenchrus clandestinus) en Australia

WILLIAM J. FULKERSON¹, NATHAN R. JENNINGS², MARK CALLOW¹, KAREN J. HARPER³, PERCY T.W. WONG⁴ AND PETER M. MARTIN⁴

¹Research and Development Office, Norco Co-operative Ltd, Lismore, NSW, Australia. norco.com.au

²North Coast Local Land Services, Lismore, NSW, Australia. northcoast.lls.nsw.gov.au

³School of Agriculture and Food Sciences, The University of Queensland, Lawes, QLD, Australia. gatton.uq.edu.au

⁴Plant Breeding Institute, University of Sydney, Cobbitty, NSW, Australia. sydney.edu.au

Abstract

While kikuyu (*Cenchrus clandestinus*) is an important grass for dairy and beef production in the subtropical region of Australia and the world, the most common cultivar, Whittet, is seriously affected by the fungal diseases, kikuyu yellows (*Verrucalvus flavofaciens*) and black spot (*Bipolaris* spp.). Thus resistance to these diseases is a priority in selecting a better kikuyu cultivar, along with higher herbage quality and yield and better winter growth. A study was conducted to identify suitable candidates from kikuyu ecotypes collected along the east coast of Australia plus lines obtained by subjecting Whittet to a mutagenic agent. Initial glasshouse studies identified 19 lines that were resistant to the KY1A strain of kikuyu yellows and 4 of these, with forage quality and yield superior to Whittet, were further evaluated in the field at 2 sites using Whittet as the control. At Site 1, line 12A demonstrated a much higher level of resistance to kikuyu yellows than Whittet, with 85% of plants resisting infection compared with only 15% of Whittet plants. At Site 2, the numbers of 12A and Whittet plants infected were similar. Further tests, using kikuyu yellows inoculum collected from 11 sites along the east coast of Australia, found that only 15% of 12A plants became infected compared with 61% of Whittet plants. Thus, kikuyu line 12A was resistant to most, but not all, strains of the kikuyu yellows pathogen. Annual yield of 12A (19,008 kg DM/ha) was 24% higher than that of Whittet and 12% higher than Acacia, but the difference was significant only for Whittet. During summer, 12A produced 10,212 kg DM/ha (24% higher than Whittet), was more active in early spring, had slightly higher dry organic matter digestibility (66.7 vs. 64.0%) and was resistant to black spot infection.

Keywords: Black spot (*Bipolaris* spp.), cow preference, forage quality, kikuyu yellows (*Verrucalvus flavofaciens*), tropical pasture.

Resumen

El pasto kikuyo (*Cenchrus clandestinus*) es una especie ampliamente utilizada para la producción de ganado de leche y carne en la región subtropical de Australia. El cultivar (cv.) Whittet es el más utilizado; no obstante es seriamente afectado por dos enfermedades fungosas, el amarillamiento de kikuyo, causado por *Verrucalvus flavofaciens*, y la mancha negra (*Bipolaris* spp.). Por tanto, la resistencia a estas enfermedades es una prioridad en el desarrollo de mejores cultivares de esta gramínea, conjuntamente con una mayor producción y calidad del pasto y mejor crecimiento en época de invierno. En New South Wales, Australia, se realizó un estudio para seleccionar líneas superiores entre ecotipos de kikuyo recolectados a lo largo de la costa este de Australia, y germoplasma que se obtuvo después de someter plantas

del cv. Whittet a un agente mutagénico. En estudios de invernadero iniciales se identificaron 19 líneas resistentes a la cepa KY1A del amarillamiento de kikuyo. Cuatro de ellas, que presentaron calidad forrajera y rendimiento superiores a cv. Whittet, se evaluaron a nivel de campo en dos sitios, usando cv. Whittet como testigo. En el Sitio 1, la línea 12A demostró un nivel más alto de resistencia al amarillamiento de kikuyo que el cv. Whittet, con 85% de las plantas resistentes a la infección, en comparación con solo 15% de las plantas del cv. Whittet. En el Sitio 2, el número de plantas infectadas de la línea 12A y cv. Whittet fue similar. En pruebas adicionales, utilizando inóculo del amarillamiento de kikuyo recolectado en 11 sitios a lo largo de la costa este de Australia, solo 15% de las plantas de la línea 12A quedaron infectadas en comparación con 61% de las plantas del cv. Whittet. Por tanto, la línea 12A fue resistente a la mayoría, pero no a todas, de las cepas del patógeno. El rendimiento anual de la línea 12A (19,008 kg MS/ha) fue 24% mayor que el del cv. Whittet y 12% mayor que el de otro cultivar comercial en Australia, cv. Acacia, pero la diferencia solo fue significativa para cv. Whittet. Durante el verano, la línea 12A produjo 10,212 kg de MS/ha (24% más que cv. Whittet), creció mejor a principios de la primavera, presentó una digestibilidad de la materia orgánica ligeramente más alta (66.7 vs. 64.0%) y fue resistente a la infección por mancha negra.

Palabras clave: Amarillamiento de kikuyo (*Verrucalvus flavofaciens*), calidad forrajera, gramínea tropical, mancha negra (*Bipolaris* spp.), palatabilidad relativa.

Introduction

Kikuyu grass (*Cenchrus clandestinus*) is a C4 summer grass, native to northeastern parts of Africa, predominantly in the Aberdare Mountain region of Kenya, which is inhabited by the Kikuyu tribe; hence the name, kikuyu. It is found at elevations between 1,950 and 2,700 m in the tropics, creating a niche subtropical climate with maximum temperature of 16–24 °C and a minimum of 2–8 °C (Morrison 1969), which led Pearson et al. (1985) to conclude that kikuyu had an optimal temperature range for growth of 8–21 °C. However, kikuyu is very adaptable, having become naturalized in areas with far more extreme temperatures, such as South Africa and Australia, where it grows successfully from the tropical highlands of the Atherton Tableland (North Queensland) to East Gippsland, Victoria (temperate climate) and the irrigated pastures of southwest Western Australia (Mediterranean climate).

'Common' kikuyu grass, introduced into Australia in 1919, was traditionally established by cuttings until Wilson (1968) identified a seeding line of kikuyu grass at Grafton Research Station in northern NSW. This line was primarily selected for its high level of free seeding and more vigorous growth than Common kikuyu grass, particularly in winter, and was registered as cultivar Whittet in 1970.

Three more lines were subsequently selected and registered:

1. 'Breakwell', selected for its ability to set seed but similar to Common kikuyu grass in other aspects, was registered in 1971.
2. 'Crofts' is claimed to have greater cold tolerance and to be slightly higher yielding than Whittet and was released in 1983.

3. 'Noonan' was selected for resistance to kikuyu yellows (*Verrucalvus flavofaciens*) (Wong and Wilson 1983) and was registered in 1983 but is now used mainly as a turf grass.
4. 'Acacia' selected from the Acacia Plateau at high elevation in the subtropical region was registered in 2013.

A survey in 1994 (Anonymous 1994) revealed that kikuyu grass was the basic forage for 30% of dairy pastures in NSW and it was estimated that 70% of milk production in summer came from kikuyu pastures. However, since then, the spread of the fungal diseases, kikuyu yellows and, to a lesser extent, black spot (*Bipolaris* spp.), inter alia, have reduced its contribution. When Mears (1970) reviewed kikuyu grass in 1970, the adverse impact of kikuyu yellows on kikuyu pastures was already evident. He concluded that "improvement (of kikuyu grass) should be directed towards disease resistance (e.g. kikuyu yellows), possibly higher digestibility and cold tolerance rather than vigour or free seeding". The dairy industry still considers these traits are top priority for selecting a better kikuyu cultivar.

Resistance/tolerance to fungal diseases

Wong (1975) found that an undescribed fungus, subsequently described as a new species, *Verrucalvus flavofaciens*, by Dick et al. (1984), caused kikuyu yellows. It is spread by water-borne zoospores which infect the roots, resulting in death of the grass in patches. No commercial fungicides are currently available to control kikuyu yellows effectively.

With black spot disease, the tips of the more mature leaves of infected kikuyu plants often turn yellow and numerous dark brown to black spots appear on the leaf

blades and sheaths, especially during periods of high humidity and rainfall. Normally, black spot does not kill the plant, but growth is slightly reduced, and more importantly, the leaves become chlorotic and unpalatable to cattle.

Tolerance of low temperatures

In the subtropical dairy region of NSW, kikuyu pastures are typically over-sown with short-rotation ryegrass in autumn to provide winter feed, when kikuyu growth is low (Fulkerson et al. 2010). This over-sowing is costly and results can be variable, dependent on weather. Finding a kikuyu genotype more tolerant of winter cold would eliminate the need to sow ryegrass or shorten the period when it was needed.

Improved forage quality

Selection for improved forage quality, specifically digestibility, is also a common dairy industry request, as a means of achieving higher milk production. While ryegrass can produce 20 L milk/cow/day with good management on-farm, Reeves (1998) found cows grazing kikuyu during mid-lactation produce about 15 L milk/day, i.e. at a time when virtually all milk is coming from feed and not body reserves.

Based on these factors, the present study aimed to identify a kikuyu line with one or more of the following desirable traits: resistance to fungal diseases, improved herbage quality, greater tolerance of winter cold and higher yield than cv. Whittet, in that order of priority..

Materials and Methods

There were 4 stages in the evaluation of available genetic material of kikuyu as outlined below:

Stage 1 - Yield, forage quality and resistance to kikuyu yellows of 115 kikuyu lines, as single plants, were evaluated.

Stage 2 - Kikuyu plants identified as resistant to kikuyu yellows in Stage 1 were tested in the field, using Whittet as the control.

Stage 3 - The kikuyu line, 12A, identified as resistant to kikuyu yellows in Stage 2, and the commercial kikuyu cultivar, Whittet, were further tested for resistance to kikuyu yellows by using inoculum from kikuyu yellows-infected plants, collected over a wide geographical region.

Stage 4 - The 2 commercial cultivars, Whittet and Acacia, plus line 12A were evaluated against 6 lines found to be highest in other desirable traits (yield and forage quality) during Stage 1.

Stage 1 – Initial screening

Material tested. The study commenced in March 2015 with the evaluation of 115 kikuyu lines, selected from 1,600 lines held at the Animal Science Precinct, University of Queensland from a previous study on the genetic variation in kikuyu grass (Lowe et al. 2010) with those lines originating from 3 different sources:

- Wollongbar collection (DAN063) - These plants originated from Whittet seed subjected to mutagenic agents by Dr D. Luckett, plant breeder, Department of Primary Industries, Agricultural Research Institute, Wagga Wagga, NSW in 1996 and initially evaluated at Wollongbar Agricultural Institute, NSW.
- Kevin Lowe collection - Kikuyu ecotypes collected from throughout Queensland.
- Peter Martin collection - Kikuyu ecotypes collected from throughout NSW.

Forage yield and quality. The plants were tested for the following:

- Dry matter yield of lines as individual potted plants at the recommended stage of regrowth (4.5 new leaves/tiller).
- Ash-free neutral detergent fiber (NDFom) and indigestible NDFom (iNDFom) (Harper and McNeil 2015) at the School of Agriculture and Food Sciences, University of Queensland, Gatton Campus, Queensland. The samples were analyzed for iNDFom using long-term (10 days) in vitro fermentation. This procedure is based on a fermentation procedure described by Goering and Van Soest (1970), adapted for use with Ankom filter bags using ANKOM Daisy incubators. Neutral detergent fiber concentration was determined using the procedure described by Goering and Van Soest (1970) and adapted for an Ankom fiber analyzer.
- Dry organic matter digestibility (DOMD) by the rumen fluid fermentation method (Tilley and Terry 1963) at the NATA-accredited feed testing laboratory, Department of Primary Industries, Charles Sturt University, Wagga Wagga, NSW.

The mean, standard deviation (SD) and range of the results for the 115 kikuyu lines were: NDFom 62.4% (± 3.8) (range 54.3–71.9%); and iNDFom 20.7% (± 4.6) (9.2–29.9%).

Resistance to kikuyu yellows infection. The sensitivity of the kikuyu plants to kikuyu yellows was tested in a glasshouse at the Plant Breeding Institute, University of Sydney, Cobbitty, NSW, using kikuyu yellows-infected kikuyu leaves as described by Wong (2011). Where there was any doubt that the yellowing symptoms were due to

the disease, yellow leaves were collected at random to re-isolate the pathogen and to serve as a check on the method. This method was also used to confirm kikuyu yellows infection in the regional samples in Stage 3. Nineteen kikuyu lines showed resistance to the KY1A strain of kikuyu yellows, one of the 3 strains previously identified by Dr P. Wong and believed to be the most prevalent.

Selected lines. Four of these kikuyu lines (Table 1), with forage quality (NDFom, iNDFom and DOMD) at least as good as Whittet, were chosen for further evaluation against Whittet, as the control, in Stage 2.

Table 1. Neutral detergent fiber (NDFom), indigestible NDFom (iNDFom), dry organic matter digestibility (DOMD) (all as % DM) and yield (g DM/plant), sampled in March 2015 from individual plants of 4 kikuyu lines and Whittet as control.

Kikuyu line/cultivar	NDFom (%)	iNDFom (%)	DOMD (%)	Yield (g DM/plant)
12A	62.0	16.4	69	10.7
11C	59.5	17.0	69	8.6
Whittet	61.9	19.7	67	8.3
15A	57.4	15.7	70	10.7
25D	61.2	14.3	68	9.3

The origins of these 5 lines were as follows:

- Lines 15A, 11C and 12A were unidentified plants, which originated from the combined Queensland and Wollongbar collections.
- Line 25D was the only identifiable plant and had shown some resistance to kikuyu yellows in the initial field evaluation at Wollongbar (Dairy Australia-funded project, DAN063).

Stage 2 - Field evaluation of kikuyu lines for resistance to kikuyu yellows

Propagation and establishment. Cultivar Whittet plus the 4 kikuyu lines selected for the field evaluation were propagated vegetatively from the original 'mother' plants by means of rooted stolons. These were planted out on 20 December 2015 in 1 m² plots, forming a grid of 5 lines of kikuyu grass with 5 plants/line, replicated 4 times at 2 locations (Site 1 – Kyogle: 28° S, 153° E; 76 masl; and Site 2 – Lismore: 29° S, 153° E; 12 masl). This grid structure facilitated weeding and helped to identify lines and prevent incursion between lines through stolon growth. The soils were clay loams on a flood-plain aspect.

The climate at both sites is subtropical with Site 1 having lower rainfall and humidity but higher summer temperature than Site 2. From planting to the completion

of the study on 30 May 2016, Site 1 received 323 mm of rain and Site 2 had 406 mm.

At planting, the areas where the plots were located, showed obvious signs of current kikuyu yellows infestation (stunted growth, yellowing of the leaves, short stolon internodes and little root development, making the plants easy to pull from the soil). The soils at both sites were highly fertile with high original soil nitrate levels (>80 mg/kg). In order to stress the plants, in an effort to maximize active kikuyu yellows infestation, supplementary irrigation was applied only for the first 4 weeks after planting to enable the plants to establish effectively and no N fertilizer was applied.

Confirmation of kikuyu yellows status. On 16 March 2016, stolon and leaf samples were taken from one 'yellow' plant of each kikuyu line in each replicate as well as from the 'green' 12A plants. All samples were tested for kikuyu yellows infection, to confirm or reject the visual symptoms, using the following methods:

- Plating out of leaf pieces on water agar plates after surface sterilization (P. Wong, unpublished data); and
- Floating leaf pieces of kikuyu in soil extract after surface sterilization ([Wong 1975](#)).

Stage 3 - Resistance to disease inoculum from the north coast of NSW and SE Queensland

In order to determine the geographical extent of the resistance of line 12A to kikuyu yellows relative to Whittet, kikuyu leaves from plants, which were obviously affected by kikuyu yellows, were collected from 11 dairy pastures located along the north coast of NSW and SE Queensland and used as a source of inoculum ([Wong 2011](#)). In this experiment, 3 replicates of each inoculum were tested against 12A and Whittet. The plants were kept in a glasshouse at 30:25 °C (day:night) temperature for about 3 months from mid-January. Kikuyu yellows infection usually became obvious after 5–7 weeks. One tiller or leaf from each plant, suspected to be infected, was tested to confirm kikuyu yellows, as outlined previously.

Stage 4 - Field evaluation of kikuyu lines for yield, forage quality, stolon vigor, black spot susceptibility and cow preference

This plot experiment was undertaken at the same location as used in Stage 2, i.e. Site 2 (Lismore). The climatic data during Stage 4 are shown in Figure 1.

Six kikuyu lines selected in Stage 1 for high quality (DOMD, NDFom and iNDFom) or yield, as individual plants were selected for evaluation in this stage (Table 2).

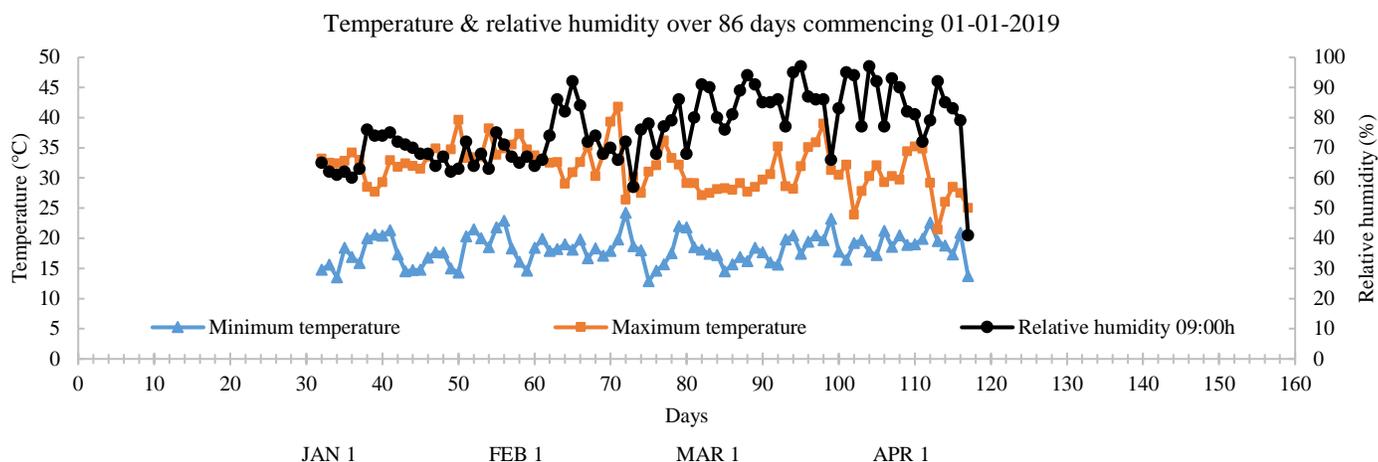


Figure 1. The minimum and maximum ambient temperatures (°C) and relative humidity (%) at 09:00 h during the experiment.

Table 2. Kikuyu lines selected in Stage 1 for high quality (DOMD, NDFom and iNDFom) or yield, as individual plants, evaluated in Stage 4 in plots.

Kikuyu line	NDFom (% DM)	iNDFom (% DM)	DOMD (% DM)	Yield (g DM/plant)
15A	57.4	15.7	70	10.7
18A	52.6	14.2	71	5.7
7A	55.9	18.4	65	11.6
14B	56.1	13.6	68	8.8
2A	57.3	9.2	68	7.7
3A	59.4	9.2	64	8.4

The commercial cultivars, Whittet and Acacia, and kikuyu line 12A, which was shown to be resistant to kikuyu yellows in Stages 2 and 3, were also included in the experiment (see Table 1 for quality and yield data).

Parameters measured were: seasonal yield, forage quality, stolon vigor, level of black spot infestation, winter growth and cow preference, in 2 × 1 m field plots, randomly allocated within 3 replicates. The evaluation continued for 11 months (24 November 2016–24 October 2017). The plants were vegetatively propagated from the original ‘mother’ plants in August 2016 and transplanted into the plots on 6 October. Recording of yield and other traits commenced with the first recorded defoliation on 8 December 2016.

Site preparation. Lime and a complete fertilizer (Rubisca plus at 350 kg/ha containing N:P:K:S at 12:5:14:9.7 mg/kg plus trace elements) were applied to the total plot area before planting to ensure that soil pH and soil nutrients [Colwell P - 110; exchangeable K - 227; S - 30 in mg/kg; pH (CaCl₂) - 5.1] were not limiting kikuyu growth. Urea was then applied after each defoliation at a rate equivalent to a per-month recommendation of 100 kg/ha.

Methodology and chemical analysis. The plots were cut to 6 cm stubble height with a Massport hand push mower each time the plants had regrown 4–4.5 new leaves/tiller (after approximately 14 days in ‘summer’ extending out to 35 days or more in ‘winter’), i.e. the stage when herbage quality and yield are optimized (Reeves 1998). The ‘green’ plot yield and weight of a ‘green’ subsample were recorded before the green subsample was dried at 65 °C for 48 h in a forced-draft oven.

At the defoliations on 27 February, 24 April and 2 December 2017, herbage samples were taken for analyses of DOMD, NDFom and iNDFom (for details see Stage 1) and the number of stolons protruding from each plot was recorded as an indication of stolon vigor.

The presence of the fungal infection, black spot, was scored as: 0 (no infection), 1 (slight infection - 4–6 yellow leaf tips/plot), 2 (moderate - more than half the leaf tips yellow) and 3 (severe - nearly all leaf tips yellow), prior to each defoliation from 4 December 2016 to 9 May 2017.

A cow preference test (Horadagoda et al. 2009) was performed 14 days after the final harvest of the experiment on 16 December 2017 (when 4 new leaves/tiller had regrown and at a time when there was no black spot infection). The test involved 2 observers recording which plots were being grazed every 10 seconds for 40 minutes by 3 Jersey milking cows, accustomed to grazing kikuyu pastures.

Results

Stage 2 - Field evaluation of kikuyu lines for resistance to kikuyu yellows

The status of the kikuyu plants at Site 1 (Kyogle) on 20 April (130 days after planting) and at Site 2 (Lismore) on 31 May (171 days after planting) is shown in Table 3.

Observations on plots at Lismore were continued until May as a number of plants were seen to be recovering in April, whereas most plants that were infected with kikuyu yellows at Kyogle had died by 20 April.

The photos below (Figure 2) show Replicate 2 at Site 1, 38 days after planting and at 130 days after planting, illustrating the impact of kikuyu yellows on the kikuyu lines.

Table 3 shows the very marked differences at Site 1 between line 12A and the other 4 kikuyu lines which had all, apart from Whittet, shown resistance to the kikuyu yellows strain KY1A in the initial glasshouse tests in Stage 1. At Site 2, the proportion of plants infected with kikuyu yellows was substantially lower than at Site 1 and all lines recorded similar levels of infection, but a high percentage of infected plants recovered in late autumn. In addition, the onset of infection was later, which suggests the strain of kikuyu yellows at Site 1 was different from that at Site 2.

At Site 1, kikuyu yellows was isolated from 19 leaf/stolon samples that displayed symptoms of kikuyu yellows infection, confirming the presence of the disease, while 2 plants of 12A, identified as ‘green’ or symptomless, were confirmed as free from kikuyu yellows infection. At Site 2, 15 of the 18 ‘yellow’ leaf/stolon samples were confirmed as infected and 2 leaves/stolons identified as ‘green’ were also infected with kikuyu yellows.

Stage 3 - Resistance to disease inoculum from the north coast of NSW and SE Queensland

In this test, only 1 or 2 plants of the 3 became infected, probably because some of the leaves originally collected and showing obvious symptoms of kikuyu yellows may not have had viable inoculum at the time of sampling. Therefore, it was not possible to say definitively at which sites 12A would be resistant to kikuyu yellows. However, the results support the proposition that 12A is far more resistant to kikuyu yellows than Whittet with only 5 plants (of 33 treated) becoming infected and 2 recovering, compared with 20 Whittet plants becoming infected, of which 6 recovered.

Stage 4 - Field evaluation of kikuyu lines for forage quality and other desirable traits

The forage quality of the 7 kikuyu lines and the 2 commercial cultivars used in this stage are shown in Table 4.

In ‘summer’, DOMD of kikuyu line 12A was 2.7% units higher than that of Whittet but the differences were not significant. The DOMD of line 18A was significantly higher than that of any other line except 3A. There were no significant differences in DOMD for the samples taken in ‘autumn’ and ‘winter-spring’.

Table 3. The kikuyu yellows status of kikuyu plants at Kyogle (Site 1) on 20 April and Lismore (Site 2) on 31 May. Values indicate no. of plants in the respective categories.

Kikuyu line/cultivar	Kyogle				Lismore ¹			
	Green	Yellow	Dead	Recovered	Green	Yellow	Dead	Recovered
12A	17	2	0	1	3	0	7	10
11C	3	3	12	2	6	4	5	5
Whittet	3	0	17	0	6	0	8	6
15A	1	3	15	1	5	1	10	4
25D	6	1	12	1	8	0	2	6

¹There were only 16 plants available for line 25D at Lismore.



Figure 2. The status of kikuyu lines in Replicate 2 at Site 1 at 38 days after planting (left) and 130 days after planting (right). Lines (from left to right) are: 12A (all green), 11C (2 dead, 2 yellow, 1 green), Whittet (all dead), 15A (4 dead, 1 yellow) and 25D (3 dead, 1 yellow).

Table 4. Dry organic matter digestibility, ash-free neutral detergent fiber and indigestible NDF (% DM) for kikuyu samples taken on the dates shown.

Kikuyu line/cultivar	27/02/2017 'Summer'	24/04/2017 'Autumn'	2/12/2017 'Winter-spring'
Dry organic matter digestibility (DOMD)			
12A	66.7bcd ¹	63.1abcd	65.9
Whittet	64.0de	62.9bcd	65.7
Acacia	66.9bc	64.0abc	66.0
15A	62.6e	59.6d	NA ²
18A	69.7a	64.7ab	66.7
7A	65.1cde	61.7cd	NA
14B	66.8bc	66.5a	NA
2A	64.0e	61.7cd	66.0
3A	68.7ab	65.7ab	67.0
Significance	P = 0.001	P = 0.022	NS
Ash-free neutral detergent fiber (NDFom)			
12A	61.0b	65.0	57.3
Whittet	62.2ab	65.0	57.0
Acacia	65.0a	66.7	57.3
15A	61.0b	NA	NA
18A	59.0b	65.0	55.9
7A	60.3b	NA	NA
14B	60.1b	NA	NA
2A	59.3b	65.0	56.7
3A	62.0b	65.7	58.8
Significance	P = 0.028	NS	NS
Indigestible NDFom (iNDFom)			
12A	20.0abc	10.5bc	14.9
Whittet	22.4a	10.0c	15.0
Acacia	18.8bcd	12.7a	14.7
15A	21.2ab	NA	NA
18A	16.7d	11.7ab	13.6
7A	20.4abc	NA	NA
14B	19.2bcd	NA	NA
2A	21.1ab	10.7bc	14.6
3A	18.4cd	10.0c	15.6
Significance	P = 0.008	P = 0.015	NS

¹Within columns and parameters means without a common letter are significantly different at the levels indicated.

²Analysis of kikuyu lines 15A (low quality and yield) and 7A and 14B (both highly affected by black spot fungus) were discontinued after the first analysis in summer owing to the issues indicated.

The NDFom of Acacia was significantly higher than that of all other kikuyu lines except Whittet in 'summer', while NDFom values of all kikuyu lines in 'autumn' and 'winter-spring' were similar.

The iNDFom in 'summer' was highest for Whittet and lowest for 18A and the difference was significant but, by contrast, the iNDFom of Acacia in 'autumn' was significantly higher than that of any other line, except 18A. There was no difference in iNDFom between lines in 'winter-spring'.

Dry matter yield. Forage yields for the 11-month period from 8 December 2016 to 24 October 2017 are shown in Table 5. Clearly, 12A was higher-yielding than both Whittet and Acacia during the main 'summer' growth period but not different from lines 2A, 7A, 18A and 14B. Line 12A also produced higher yields than Whittet in the 'winter-spring' period but the difference was not significant ($P > 0.05$). Total yield for 12A was significantly greater than that for Whittet but not for Acacia nor lines 2A, 7A and 18A.

Table 5. Dry matter yields during 'summer', 'autumn' and 'winter-spring' and total yield for the 7 kikuyu lines (including 12A) and the 2 commercial cultivars, Whittet and Acacia.

Kikuyu line/cultivar	Dry matter yield (kg DM/ha)			
	24/11 to 29/3 'Summer'	30/3 to 9/5 'Autumn'	10/5 to 24/10 'Winter/spring'	Total
12A	10,212a ¹	1,396	7,480	19,088a
Whittet	8,226bc	1,245	5,963	15,434c
Acacia	8,258bc	1,460	7,283	17,001abc
15A	8,343bc	1,114	5,934	15,390c
18A	9,126ab	1,355	6,473	16,954abc
7A	9,397ab	1,500	7,040	17,937abc
14B	8,841abc	1,382	6,169	16,391bc
2A	9,481ab	1,585	8,260	19,327a
3A	7,553c	1,352	6,456	15,360c
	P = 0.024	NS	P = 0.1	P = 0.05

¹Within columns means without a common letter are significantly different at the levels indicated.

Other desirable traits. The incidence of black spot and indications of stolon vigor for 12A, Whittet and Acacia were recorded during the 'summer' and 'autumn' periods from 22 December 2016 to 9 May 2017, while the cow preference test was recorded in early December 2017, when there were no symptoms of black spot infection and none was expected owing to the low relative humidity at that time (Table 6).

Black spot infestation on 12A was zero and on Acacia was low but the mean score for Whittet was 1.6, being between moderate and severe at each defoliation over the 'summer' growth period and this difference was significant.

Whittet showed the highest stolon vigor, being more than 70% greater than 12A and nearly 3 times as great as Acacia but these differences were not significant ($P > 0.05$). While line 12A was preferred by cattle over both Whittet and Acacia, differences were again not significant, but did indicate that 12A was not less palatable than the cultivars at this stage of growth.

The germination rate of line 12A and the cultivars, Whittet and Acacia, were all over 80% and seedling vigor was similar.

Table 6. Mean score for incidence of black spot fungal infection during the 'summer-autumn' growth period, stolon vigor and cow preference.

Kikuyu line/ cultivar	Black spot ¹ (score)	Stolon vigor ² (No./harvest)	Cow preference ³ (recordings)
12A	0	11.7	17
Whittet	1.6	20.1	11
Acacia	0.1	6.8	5
Significance	P<0.009	NS	NS

¹0 = no infection; 1 = slight infection, 4–6 leaves/plot were yellow; 2 = moderate, more than half the leaf tips were yellow; and 3 = severe, nearly all leaf tips were yellow; recorded during the experimental period.

²Mean number of stolons protruding outside the plots at a defoliation; recorded during the experimental period.

³Recorded grazings in 40 minutes at 10-second intervals; at the end of the experiment.

Discussion

In this study, a line of kikuyu grass, 12A, was identified as being more resistant to the fungal diseases kikuyu yellows and black spot and higher yielding than the cultivars Whittet and Acacia and similar to the two commercial cultivars for stolon vigor, herbage quality and cow preference. The most important attribute, where 12A was superior to the other lines, was resistance to the fungal disease, kikuyu yellows, in most, but not all cases. The resistance to kikuyu yellows differed markedly between the 2 evaluation sites. At Site 1, the resistance was outstanding, with only 15% of 12A plants infected with kikuyu yellows, whereas over 85% of plants in the other lines, including Whittet, were infected and all infected plants died. This was despite the fact that all lines, except Whittet, were found to be resistant to the KY1A strain of kikuyu yellows, when individual plants were tested under glasshouse conditions in Stage 1 of the study.

In contrast, at Site 2, line 12A showed no advantage over the other kikuyu lines under test in terms of resistance to kikuyu yellows with 85% of plants infected. However, 49% of all plants infected with kikuyu yellows in summer recovered in the autumn, compared with only 8% at Site 1. This was despite the fact that the minimum temperature remained above 15 °C longer than normal and well into April (Table 7), a temperature conducive to kikuyu yellows activity. In addition, the onset of visual symptoms of kikuyu yellows infection was later at Site 2 than at Site 1. This difference between sites was presumably due to strain differences of the pathogen at each site with the strain at Site 1 being more pathogenic.

The regional study also supports the contention that line 12A has greater resistance to kikuyu yellows than Whittet with only 16% of 12A plants becoming infected compared

with 64% for Whittet and nearly all inoculum that infected 12A plants came from 2 properties on the lower north coast of NSW. In addition to displaying resistance to kikuyu yellows, 12A also had significantly higher 'summer' and total yields than Whittet plus earlier onset of growth in early spring with dry matter yields of 12A and Acacia being twice that of Whittet at the first spring defoliation. The results also indicate that forage quality, stolon vigor and cow preference for 12A are at least as good as those for the 2 commercially-available kikuyu cultivars.

Table 7. The long-term mean minimum ambient temperature for Site 2 (Lismore) from January to April and the actual minimum temperature in 2016 (data for Site 1 at Kyogle were not available).

Month	Average Min. temp. (°C)	2016 Min. temp. (°C)
January	18.7	17.4
February	18.6	18.8
March	17.0	17.7
April	14.1	16.6

The complete absence of black spot infection in 12A, compared with a very high level of infection in Whittet and other lines tested in Stage 4, is also very important. Unlike kikuyu yellows, black spot does not kill the kikuyu plant and scarcely affects its growth, as only the tips of the mature leaves are affected, but palatability of the infected herbage for grazing cattle is very low. Thus, dairy farmers consider that black spot is just as important a problem as kikuyu yellows.

Interestingly, the DOMD for samples of 12A were not significantly different from those of Whittet in any season. In this study, DOMD was assessed by the Tilley and Terry method (Tilley and Terry 1963), which is considered to most accurately reflect the availability of nutrients to the ruminant animal. One can be confident that forage from 12A was not inferior to that of Whittet.

We used 4.5 new leaves/tiller as the optimal time to defoliate, as digestibility is considered to be at a maximum at this point, after which the oldest leaves sequentially begin to senesce and the proportion of stolon:leaf growth increases (Reeves et al. 1996). In this environment, 4.5 leaves is usually reached at about 2-weeks regrowth in 'summer' and 3–4-weeks regrowth in late 'spring and early 'autumn' and is almost entirely dependent on ambient temperature.

While the DM yield of 12A over the main 'summer' period was greater than those of both Whittet and Acacia plus 15A and 3A, total DM yield of 12A over the 11 months was greater than those of Whittet, 15A, 3A and 14B only. The observation that leaves of Whittet were yellow during 'winter', while 12A and Acacia remained green, was in keeping with the higher yields of 12A and Acacia at the first defoliation after 'winter' on 6 October.

This was not unexpected for Acacia, as it was selected from the Acacia plateau at a higher elevation and in a colder climate. This ‘winter’ activity is a highly desirable trait for a dairy pasture and more particularly for beef farms, where over-sowing of kikuyu with ryegrass in autumn, to provide winter feed, is not practiced often, primarily due to cost.

Conclusions

This study has identified a kikuyu line, 12A, which appears superior to the commercial cultivars Whittet and Acacia, in terms of resistance to fungal infection, i.e. kikuyu yellows, in most but not all situations, and *Bipolaris* spp., both of which have been responsible for a major decline in the area of kikuyu pastures grown in the subtropical dairy region of Australia and a hesitancy by farmers to re-establish kikuyu grass with the existing cultivars. This successful selection of 12A has been achieved along with an increase in forage yield over that of the most common commercial cultivar, Whittet, and, most importantly, without a decline in forage quality and cow preference. The study has also given indications of the genetic diversity in kikuyu grass for the traits tested. Evaluation of this line under grazing to assess the benefits of improved disease resistance and yields in terms of increased livestock production would provide further evidence for recommending widespread plantings.

Acknowledgments

The authors appreciate the financial and in-kind support provided by the Norco Co-operative Ltd, North Coast (of NSW) Local Land Services, Department of Primary Industries, NSW and the Department of Agriculture, Food and Fisheries, Queensland. The project was managed by the far north coast (of NSW) Dairy Industry Group and the authors thank the members of the group, particularly the chairperson, Leigh Shearman, and the deputy chairperson, Ken Bryant, for their support and interest in the project.

We sincerely thank also Greg and Todd McNamara and Paul Arber for making their properties available for the conduct of the field evaluations and for their interest in and enthusiasm for the project.

References

(Note of the editors: All hyperlinks were verified 10 January 2020.)

Anonymous. 1994. Survey of kikuyu in dairy pasture. NSW Agriculture, Wollongbar Research Station, Wollongbar, NSW, Australia.

- Dick MW; Wong PTW; Clark G. 1984. The identity of the oomycete causing 'Kikuyu Yellows' with a re-classification of the downy mildews. *Botanical Journal of the Linnean Society* 89:171–197. doi: [10.1111/j.1095-8339.1984.tb01008.x](https://doi.org/10.1111/j.1095-8339.1984.tb01008.x)
- Fulkerson B; Griffiths N; Sinclair K; Beale P. 2010. Milk production from kikuyu grass based pastures. Primefact 1068, Department of Primary Industries, Orange, NSW, Australia. bit.ly/39VFjOF
- Goering HK; van Soest BJ. 1970. Forage fibre analysis (apparatus, reagents, procedures and some applications). *Agriculture Handbook No 379*. Agricultural Research Services, US Department of Agriculture, Washington, DC, USA. bit.ly/3oWSPch
- Harper KJ; McNeil DM. 2015. The role of iNDF in regulation of feed intake and the importance of its assessment in subtropical ruminant systems. *Agriculture* 5:778–790. doi: [10.3390/agriculture5030778](https://doi.org/10.3390/agriculture5030778)
- Horadagoda A; Fulkerson WJ; Nandra KS; Barchia IM. 2009. Grazing preferences by dairy cows for 14 forage species. *Animal Production Science* 49:586–594. doi: [10.1071/EA08299](https://doi.org/10.1071/EA08299)
- Lowe KL; Bowdler TM; Sinclair K; Holton TA; Skabo SJ. 2010. Phenotypic and genotypic variation within populations of kikuyu (*Pennisetum clandestinum*) in Australia. *Tropical Grasslands* 44:84–94. bit.ly/3oVL1r3
- Mears PT. 1970. Kikuyu (*Pennisetum clandestinum*) as a pasture grass – a review. *Tropical Grasslands* 4:139–152. bit.ly/2XSlvr6
- Morrison J. 1969. Grasses and legumes at high altitude of tropical Africa. *Herbage Abstracts* 39:101.
- Pearson CJ; Kemp; Kirby AC; Launder TE; Miedel C. 1985. Responsiveness of seasonal temperature and nitrogen amongst genotypes of kikuyu, paspalum and Bermuda grass pastures of coastal New South Wales. *Australian Journal of Experimental Agriculture* 25:109–116. doi: [10.1071/EA9850109](https://doi.org/10.1071/EA9850109)
- Reeves M. 1998. Milk production from kikuyu grass (*Pennisetum clandestinum*) pastures in relation to season and stage of growth in a subtropical environment. Ph.D. Thesis. University of Sydney, NSW, Australia.
- Reeves M; Fulkerson WJ; Kellaway RC. 1996. Forage quality of kikuyu: The effect of time of defoliation and nitrogen fertiliser application and in comparison with perennial ryegrass. *Australian Journal of Agricultural Research* 47:1348–1359. doi: [10.1071/AR9961349](https://doi.org/10.1071/AR9961349)
- Tilley JMA; Terry RA. 1963. A two-stage technique for in vitro digestion of forage crops. *Grass and Forage Science* 18:104–111. [10.1111/j.1365-2494.1963.tb00335.x](https://doi.org/10.1111/j.1365-2494.1963.tb00335.x)
- Wilson GPM. 1968. New kikuyu variety performs well at Grafton. *Agricultural Gazette of NSW* 79:51.
- Wong PTW. 1975. Kikuyu yellows - a disease caused by an undescribed phycomycete. *Plant Disease Reporter* 59:800–801.
- Wong PTW. 2011. Screening of kikuyu selections and hybrids for resistance to kikuyu yellows. *Horticulture Australia*, North Sydney, NSW, Australia.

Wong PTW; Wilson GPM. 1983. Reaction of kikuyu cultivars and breeding lines to kikuyu yellows disease. *Australasian Plant Pathology* 12:47–48. doi: [10.1071/APP9830047](https://doi.org/10.1071/APP9830047)

(Received for publication 22 July 2020; accepted 26 November 2020; published 31 January 2021)

© 2021



Tropical Grasslands-Forrajes Tropicales is an open-access journal published by *International Center for Tropical Agriculture (CIAT)*, in association with *Chinese Academy of Tropical Agricultural Sciences (CATAS)*. This work is licensed under the Creative Commons Attribution 4.0 International ([CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)) license.