Invasive Plant Science and Management

www.cambridge.org/inp

Identifying the geographic origins of invasive *Megathyrsus maximus* in the United States using molecular data

John F. Gaskin[1], John A. Goolsby[2], Marie-Claude Bon[3], Massimo Cristofaro[4] and Paul-André Calatayud[5]

[1]U.S. Department of Agriculture, Agricultural Research Service, Sidney, MT, USA; [2]U.S. Department of Agriculture, Agricultural Research Service, Edinburg, TX, USA; [3]European Biological Control Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Montferrier le Lez, France; [4]Biotechnology and Biological Control Agency (BBCA onlus), Rome, Italy and [5]International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya; IRD, CNRS, Université Paris-Saclay, UMR Évolution, Génomes, Comportement et Écologie, Gif-sur-Yvette, France

Research Article

Cite this article: Gaskin JF, Goolsby JA, Bon M-C, Cristofaro M, and Calatayud P-A (2022). Identifying the geographic origins of invasive *Megathyrsus maximus* in the United States using molecular data. Invasive Plant Sci. Manag. doi: 10.1017/inp.2022.7

Received: 2 November 2021
Revised: 7 January 2022
Accepted: 12 February 2022

Associate Editor:
Marie Jasieniuk, University of California, Davis

Keywords:
AFLP; biological control; cpDNA sequencing; Guineagrass; *Urochloa maxima*

Author for correspondence:
John F. Gaskin, U.S. Department of Agriculture, Agricultural Research Service, Sidney, MT 59270. (Email: john.gaskin@usda.gov)

Abstract

Megathyrsus maximus is nonnative in the neotropics, with a tall form that is commonly used as a forage grass and a smaller-statured form that is considered invasive in south Texas, USA. Biological control researchers are challenged to find an agent that will attack the short form, but not the desirable tall form in other parts of the neotropics. We conducted molecular analyses on 155 *Megathyrsus maximus* samples from its native range in Africa and compared them with U.S. short-form samples to help determine the geographic origins of its invasion. We found eight distinct genotypes in 34 short-form samples from Texas and Florida, USA. The highest genetic similarity of invasive samples was with plants from South Africa, while highest matches for the desirable tall form were from Kenya, Uganda, Ivory Coast, and Zambia. Ongoing biological control agent exploration and research has found agents from Kenya that are associated with an *M. maximus* genotype not well matched to the invasive short form, thus leading to a lack of rearing success. Two eriophyoid mite agents from the genetic match locality in South Africa have been evaluated but are not sufficiently host specific, as they develop on both the short and tall forms. Additional exploration is needed at the genetic match populations in South Africa to discover and evaluate potential biological control agents for the invasive form of *M. maximus*.

Introduction

Guineagrass [*Megathyrsus maximus* (Jacq.) B.K. Simon and S.W.L. Jacobs] is a large-statured tropical grass that has been planted around the world for use as livestock forage (Burton et al. 1973). Native to intertropical and southeast Africa, it is now considered an invasive species in parts of Asia, North and South America, Australia, and many tropical islands (CABI 2021) due to its ability to shade and outcompete other species (Rhodes et al. 2021). In subtropical southern Texas, it is implicated in the long-term declines of ground-dwelling granivorous birds such as quail and the facilitation of cattle fever tick [*Rhipicephalus microplus* (Canestrini)] invasion (Esteve-Gassent et al. 2014; Mercadier et al. 2009; Vacek et al. 2021).

Megathyrsus maximus reproduces primarily through apomixis, but some diploid and perhaps tetraploid sexual strains exist (Kaushal et al. 2015; Nakajima et al. 1979), and it forms an agamic complex with *Megathyrsus infestus* (Andersson) B.K. Simon & S.W.L. Jacobs (syn.: *Panicum infestum* Andersson) and *Panicum trichocladum* K. Schum (Muir and Jank 2004). Hybrids of these species are found in East Africa (Savidan and Pernès 1982), and perhaps elsewhere, and have made taxonomic identification difficult (Clayton and Renvoize 1982). *Megathyrsus maximus* has been assigned multiple scientific names over the years (see Rhodes et al. 2021), and the taxonomic confusion has been compounded by introductions from multiple African sources and approximately 35 cultivars listed in the Tropical Forages website (Cook et al. 2005). Both tall and short forms of *M. maximus* exist, with 28 and 7 cultivars listed for each, respectively. The tall form is generally 50% larger in plant height and leaf size and occurs in wetter and shadier conditions than the short form. The size differences may be due to hybridization between genotypes or differences in ploidy level (Rhodes et al. 2021). In the southern United States and the neotropics, the short form of the species (abbreviated as *SMm* in this study) is considered invasive, while the tall form (*TMm*) is utilized as a forage grass, mostly in the neotropics (Rhodes et al. 2021; Soti et al. 2020). The short form is considered invasive in Texas, USA, because it may better tolerate drought and can form dense stands in open pastures and disturbed areas, suppressing or displacing local plants on fertile soils in

Management Implications

The results of this genetic analysis identify native range origins of invasive *Megathyrsus maximus* (Guineagrass) in south Texas, potentially enabling identification of highly host-specific biological control agents that will attack the invasive short form of *M. maximus* but will not attack the desirable tall form that is a valuable forage in the neotropics.

pastures (Rhodes et al. 2021). Control options for invasive *M. maximus* include chemical applications, livestock grazing, prescribed fire, mechanical methods, and bioherbicides, but these are largely ineffective (Rhodes et al. 2021). Classical biological control is also proposed for *M. maximus* but would require agents specific enough to manage the invasive short form but not cause significant damage to the tall form that is used as forage, especially in Mexico and Central America. Understanding the genetic diversity of the target weed in its native and introduced ranges is critical to finding the best adapted and most host-specific agents (Goolsby et al. 2006b; Sutton et al. 2019).

It is possible that biological control agents of *M. maximus* may not be host specific enough to attack only one form and not the other, but arthropod and microbial biological control agents of weeds can be, in some cases, specific below the host-species level: for example, leaf galling mites on the fern *Lygodium microphyllum* (Cav.) R. Br. (Goolsby et al. 2006a), leafhopper insects (Cicadellidae) on Gulf coast cordgrass (*Spartina alterniflora* Loisel.) (Garcia-Rossi et al. 2003), and the rust fungus *Puccinia chondrillina* Bubak & Sid on rush skeletonweed (*Chondrilla juncea* L.) (Burdon et al. 1981). The goal of our work is to identify locations in the native range (Africa) that contain plants that are most similar genetically to the invasive short form of *M. maximus* found in the United States. These plants should be examined in the native range as a source of highly host-specific biological control agents.

Materials and Methods

We obtained fresh, silica-dried leaf material from 198 *Megathyrsus* plants from Florida and Texas in the United States ($n = 34$ short form; $n = 9$ tall form) and Africa ($n = 155$; Figure 1). All U.S. plants were weedy accessions from pastures, ditches, or roadway edges. The African populations were collected randomly from batches along roads or ways, and the species identification of the plant was confirmed by comparison with specimens from the East African herbarium in Nairobi and by using the key of Maitland and Hubbard (1927). Due to uncertainty in distinguishing tall and short forms in Africa, we did not morphologically identify at the subspecific level. We selected one disease-free young leaf from each plant, and plants sampled were at least 5 m apart. We performed DNA sequencing ($n = 144$) and amplified fragment length polymorphism (AFLP) analysis ($n = 198$) on these plants (Supplementary Material, Data File 1). We extracted genomic DNA from approximately 20 mg of silica-dried leaf material using a modified hexadecyltrimethylammonium bromide (CTAB) method (Hillis et al. 1996). For DNA sequencing we followed Gaskin and Wilson (2007). We sequenced the chloroplast *tRNA-Leu* (*trnL*) gene and *trnL-trnF* intergenic spacer using the primer pair "c" and "f" of Taberlet et al. (1991). All unique sequences (GenBank accessions MT327722 to MT327739) were aligned with Clustal W (980-bp length alignment) and constructed into a bootstrap (1,000 repeats) consensus phylogenetic tree using maximum parsimony under default parameters, all using MEGA X (Kumar et al. 2018). Our AFLP method followed Vos et al. (1995) with modifications as in Gaskin and Kazmer (2009). All 15 selective primer combinations of *Mse*I + CAA, CAC, CAT, CTA, or CTA and *Eco*RI + AAG, ACC, or ACT were prescreened for PCR product quality and number of variable loci using eight plant samples, and the two most polymorphic primer pairs were chosen (*Mse*I + CAC/*Eco*RI + ACC and *Mse*I + CAC/*Eco*RI + ACT). We generated all AFLP data on an Applied Biosystems 3130 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA 02451, USA), and omitted any individuals that did not produce a typical electropherogram pattern (i.e., noise >20 relative fluorescence units or failure to produce a sufficient number of peaks). We used NTSYS-pc v. 2.1 software (Rohlf 1992) (SIMQUAL program) to calculate the Dice (1945) similarity coefficient. Principal coordinates analysis (PCoA) was performed on Dice similarity coefficients using the DCENTER and EIGEN modules of NTSYS.

Results and Discussion

Materials from sites in the United States (nonnative) and African (native) ranges were genetically characterized with DNA sequences from the *trnc-f* cpDNA region in an attempt to find the geographic origins of this invasion in the United States. Eighteen haplotypes were recovered (Figure 2; Supplementary Material, Data File 2), with haplotype H1 found broadly from East Africa to South Africa providing a match for the short form (*SMm*) from Texas and Florida. The tall-form (*TMm*) haplotypes (H2 in Texas; H6 and H8 in Florida) were found across a broader range in Africa. *SMm* and *TMm* haplotypes differed by one to seven mutations, with H1 and H6 being most similar (Figure 2). Seven additional accessions were obtained and sequenced earlier by the European Biological Control Laboratory (EBCL) (Bon et al. 2011) for the *trnL-trnF* intergenic spacer only (436 bp of the larger region that we sequenced; Supplementary Material, Data File 2), and accessions from Mali, Central African Republic, Cameroon, Benin, and French Guiana (South America) were identical to H8, and accessions from Djibouti and Mozambique were identical to H1, H2, H6, H7, H9, and H14 for this shorter intergenic region.

To develop a higher-resolution estimate of the closest African match for the U.S. samples, we used 68 variable AFLP loci. For the U.S. samples, we found 8 *SMm* AFLP genotypes in 34 samples and 3 *TMm* AFLP genotypes in 9 samples (Figure 3). The range of genetic similarity (AFLPs) between *TMm* and *SMm* was from 40% to 69%. Similarity within U.S. *SMm* was 68% to 100%, and 60% to 100% within U.S. *TMm*. Genetic similarity of African plants to *SMm* ranged from 0% to 81%. Plants that were most similar to *SMm* (≥80%) were from Mkuze and Durban, South Africa. Similarity of African plants to *TMm* ranged from 0% to 85%. Plants that were genetically (AFLP) most similar to *TMm* (≥80%) were from Kenya, Uganda, Ivory Coast, and Zambia. Highest similarities were from Kenya (85%) (Supplementary Material, Data File 3). Variation in ploidy (e.g., Collins and Müller-Schärer 2012), as well as nuclear DNA content in plants of the same ploidy (e.g., Hopkins et al. 1996), can potentially alter phenotype, life form, and/or demographics of plants. These differences may affect biological control efficacy, thus it would be wise to investigate the chromosome numbers and DNA content of invasive *M. maximus* accessions and their genetically similar accessions from Africa.

https://doi.org/10.1017/inp.2022.7 Published online by Cambridge University Press

Figure 1. Map positions of the *Megathyrsus* samples collected in sub-Saharan Africa. Country codes are followed by number of samples collected. Red circles indicate ≥80% genetic match (amplified fragment length polymorphism [AFLP]) to U.S. short-form *Megathyrsus maximus* (*SMm*); blue circles indicate ≥80% match to U.S. tall-form *M. maximus* (*TMm*). No circle indicates <80% match to U.S. samples. Country codes: BE, Benin; BT, Botswana; CR, Cameroon; DC, Democratic Republic of Congo; ET, Ethiopia; GH, Ghana; IC, Ivory Coast; KY, Kenya; MZ, Mozambique; NI, Nigeria; SA, South Africa; TA, Tanzania; TO, Togo; UG, Uganda; ZI, Zimbabwe; ZM, Zambia.

The chloroplast sequences are useful to unravel interspecific divergences but are not fine-scale enough to distinguish origins of U.S. genotypes. As we found the invasive *SMm* cpDNA haplotype in seven African countries, we used AFLPs to obtain a higher resolution of genetic diversity and narrow down origins of *SMm* to South Africa. Multiple AFLP genotypes of both *SMm* and *TMm* in the United States confirm that multiple introductions have occurred for both forms, or multiple genotypes in fewer introductions, and point to the need to carefully evaluate each form or cultivar for invasive potential before introduction for agriculture. Though we did not perform in-depth population sampling, we did sample multiple plants ($n = 3$) at nine U.S. *SMm* locations; within four of these locations, the plants had identical AFLP genotypes (see pairwise Dice values, Supplementary Material, Data File 3), suggesting clonal reproduction. The remaining *SMm* locations contained multiple genotypes, suggesting that there is some outcrossing occurring, or multiple genotypes were introduced; this does not exclude clonal reproduction such as tillering. Outcrossing is well documented in *M. maximus* (Nakajima et al. 1979); hybridization has been extensively used in breeding programs; and there is evidence of reproductive plasticity, including facultative apomixis (see Rhodes et al. 2021). This complex situation suggests that the predominant reproductive mode of the invasion should be further investigated with more in-depth sampling, ploidy analysis, and fine-scale genetic analysis. Knowledge of a plant's reproductive mode can help determine which guild of biological control agents will be most effective at significantly impacting the physiology of this invasive grass.

Populations in Africa that contain *SMm* genotypes most similar to those in the United States (two localities in South Africa; Figure 1) should be more intensively surveyed for potential coevolved biological control agents. Potential agents should also be assessed for efficacy on all U.S. *SMm* genotypes and for lack of significant development of or damage to U.S. *TMm* genotypes identified in this study. This fine-scale identification of origins helps identify coevolved natural enemies that may be safer and more efficacious biological control agents (Gaskin et al. 2011; Harms et al. 2020).

Three candidate agents have undergone preliminary screening on *SMm* and *TMm* and the closely related North American grass, switchgrass (*Panicum virgatum* L). A stem-boring moth (*Buakea kauae* Moyal), known only from *SMm* in Kenya was imported, but researchers attributed the lack of success in rearing this agent to the genetic mismatch between *SMm* in Kenya and Texas, USA (Vacek et al. 2021). Two eriophyoid mites collected from *SMm*, *Abacarus* sp. (Eriophyidae) from Mpala, Kenya, and *Diptacus* sp. (Diplomatidae) from Hluhluwe, South Africa (a high genetic match of 79% with *SMm* from Texas), were imported, but both were able to develop on *SMm* and *TMm* (JAG, unpublished data), so are not specific enough to be used as biological control agents of the *SMm* form of *M. maximus*. Additional exploration is needed in South Africa and other places in Africa that closely match *SMm*

https://doi.org/10.1017/inp.2022.7 Published online by Cambridge University Press

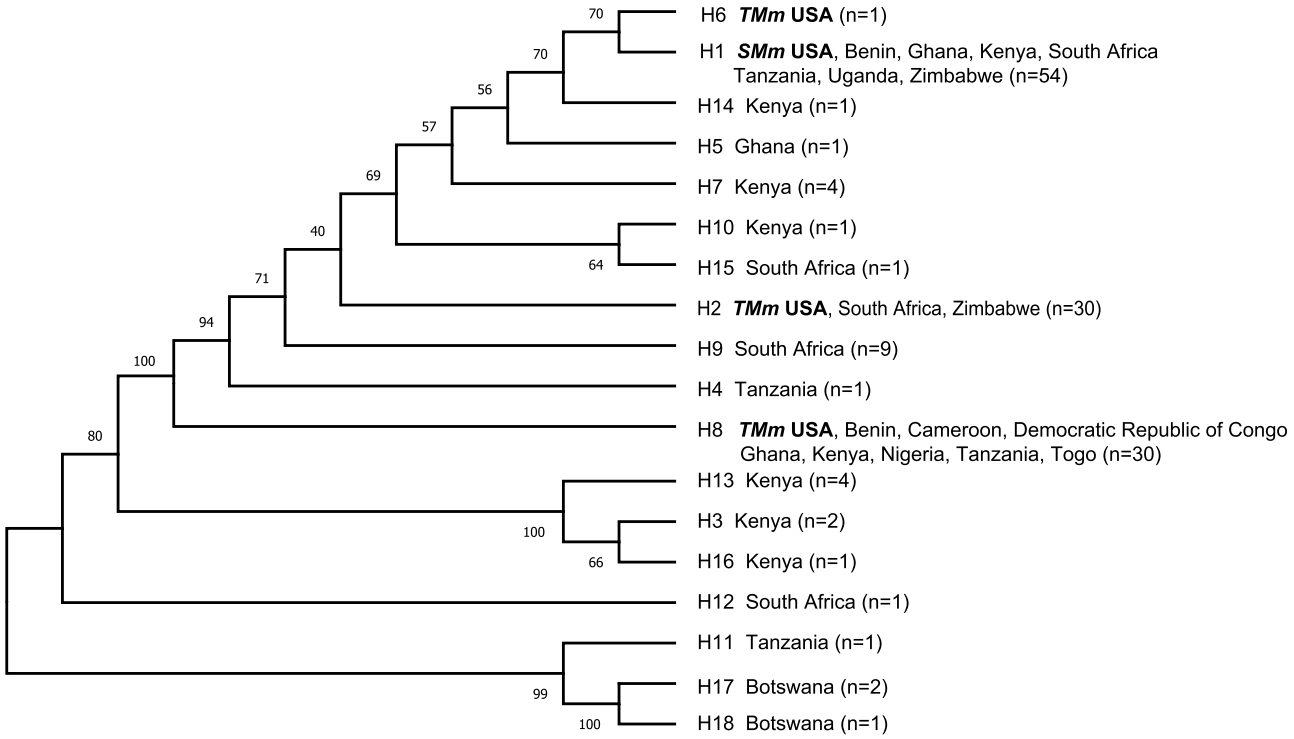

Figure 2. Consensus maximum parsimony phylogeny of 18 *trnc-f* chloroplast region DNA haplotypes (980 bp) found in 144 *Megathyrsus* samples from United States and Africa. Bootstrap values (1,000 replicates) are shown next to the branches. *SMm*, short-form *Megathyrsus maximus*; *TMm*, tall-form *M. maximus*.

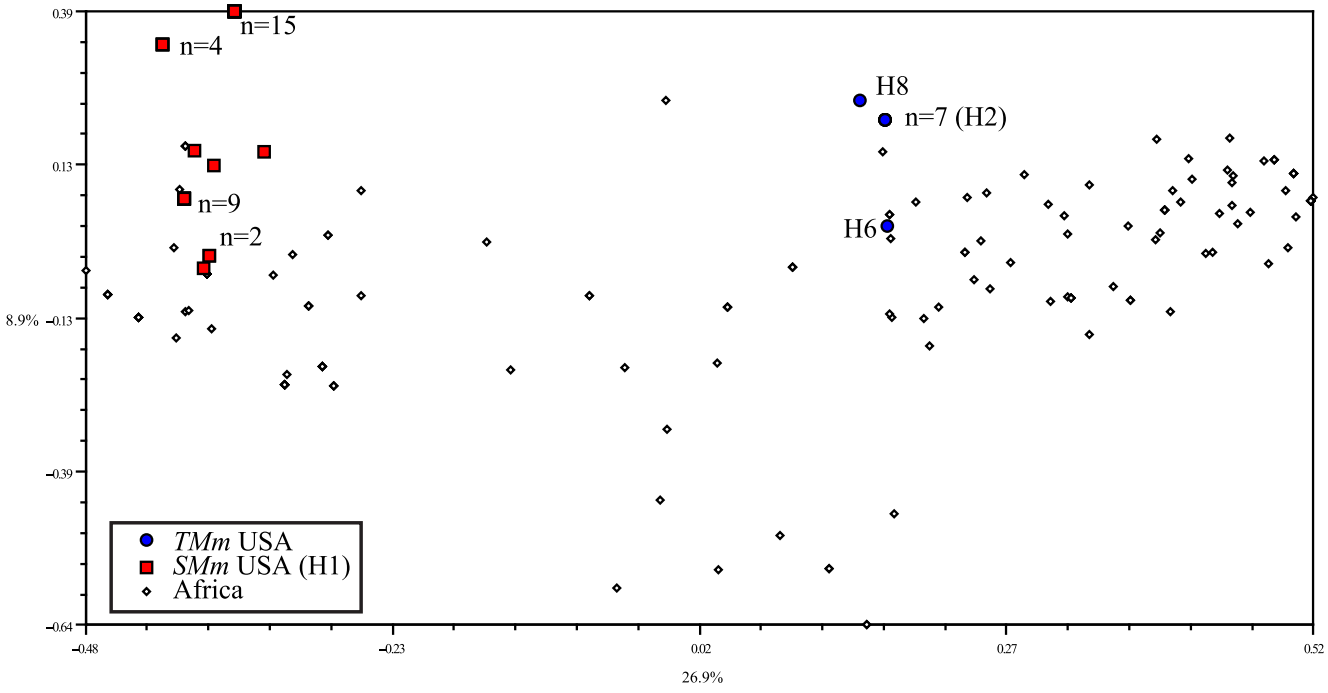

Figure 3. Principal coordinates analysis (PCoA) of amplified fragment length polymorphism (AFLP) data from 68 polymorphic loci for 198 U.S. and African *Megathyrsus* samples. If not indicated otherwise for tall-form *M. maximus* (*TMm*) or short-form *Megathyrsus maximus*, $n = 1$. Chloroplast DNA haplotypes (e.g., H1) are indicated for U.S. AFLP genotypes (red and blue symbols).

genotypes to discover and evaluate potential biological control agents of invasive *M. maximus* in the United States.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/inp.2022.7

Acknowledgments. We acknowledge the financial support of Lee and Ramona Bass Foundation, which enabled the foreign exploration and Africa-wide collections of *M. maximus* for genetic characterization. We would like to give special thanks to all plant collectors; Alan Kirk and Guy Mercadier (EBCL), Georg Goergen (IITA, Benin), Rose Ndemah (IITA, Cameroon), Onésime Mubenga

(University of Kisangani, Democratic Republic of Congo), Eric Ntiri (Togo), Régis Babin (CIRAD, Ivory Coast), Tomás Chiconel, Francisco Munguambe and Domingos Cugala (University of Eduardo Mondlane, Mozambique), Johnnie Van Den Berg (North West University, South Africa), Bonoukpoè M. Sokame and Boaz Musyoka (*icipe*, Kenya), Richard Molo (National Agricultural Research Laboratories, Uganda), Muluken Goftishu Muleta (Haramaya University, Ethiopia), Chapwa Kasoma and Gilson Chipabika (Zambia Agricultural Research Institute, Zambia), Reyard Mutamiswa and Casper Nyamukondiwa (Botswana International University of Science and Technology, Botswana), Francesca Marini and Francesca di Cristina (BBCA, Rome, Italy). We thank Iain Paterson and Guy Sutton (Rhodes University, Grahamstown, South Africa) for their input on this project, and we also thank Melanie Jeanneau (EBCL) and Kim Mann and Jeannie Lassey (USDA-ARS Sidney, MT, USA) for DNA sequencing and AFLP data. This article reports results of research only, and mention of a proprietary product does not constitute an endorsement or recommendation by the USDA for its use. USDA is an equal opportunity provider and employer. No conflicts of interest have been declared.

References

Bon MC, Goolsby J, Mercadier G, Le Bourgeois T, Poilecot P, Jeanneau M, Kirk A (2011) What do chloroplast sequences tell us about the identity of Guinea grass, an invasive Poaceae in the southern United States? Page 322 *in* Proceedings of the XIII International Symposium on Biological Control of Weeds, September 11–16, 2011, Waikoloa, USA. Wu Y, Johnson T, Sing S, Raghu S, Wheeler G, Pratt P, Warner K, Center T, Goolsby J, Reardon R, eds. Honolulu; HI: Forest Health Technology Enterprise Team

Burdon JJ, Groves RH, Cullen JM (1981) The impact of biological control on the distribution and abundance of *Chondrilla juncea* in south-eastern Australia. J Appl Ecol 18:18957–966

Burton GW, Millot JC, Monson WG (1973) Breeding procedures for *Panicum maximum* Jacq. suggested by plant variability and mode of reproduction. Crop Sci 13:717–720

[CABI] Centre for Agriculture and Bioscience International (2021) Datasheet: *Megathyrsus maximus* (Guinea grass). https://www.cabi.org/isc/datasheet/38666. Accessed: October 10, 2021

Clayton WD, Renvoize SA (1982) Graminae (Part 3). Pages 1–898 *in* Polhill RM, ed. Flora of Tropical East Africa. Rotterdam: Balkema

Collins AR, Müller-Schärer H (2012) Influence of plant phenostage and ploidy level on oviposition and feeding of two specialist herbivores of spotted knapweed, *Centaurea stoebe*. Biol Control 60:148–153

Cook BG, Pengelly BC, Brown SD, Donnelly JL, Eagles DA, Franco MA, Hanson J, Mullen BF, Partridge IJ, Peters M, Schultze-Kraft R (2005) Tropical forages. Brisbane, Australia: CSIRO, DPI&F(Qld), CIAT and ILRI https://www.tropicalforages.info/text/intro/index.html. Accessed: October 10, 2021

Dice L (1945) Measures of the amount of ecologic association between species. Ecology 26:297–302

Esteve-Gassent MD, Pérez de León AA, Romero-Salas D, Feria-Arroyo TP, Patino R, Castro-Arellano I, Gordillo-Pérez G, Auclair A, Goolsby J, Rodriguez-Vivas RI, Estrada-Franco JG (2014) Pathogenic landscape of transboundary zoonotic diseases in the Mexico–US border along the Rio Grande. Front Public Health 2:1–23

Garcia-Rossi D, Rank N, Strong DR (2003) Potential for self-defeating biological control? Variation in herbivore vulnerability among invasive *Spartina* genotypes. Ecol Appl 13:1640–1649

Gaskin J, Kazmer D (2009) Introgression between invasive saltcedars (*Tamarix chinensis* and *T. ramosissima*) in the USA. Biol Invasions 11:1121–1130

Gaskin JF, Bon MC, Cock MJ, Cristofaro M, De Biase A, De Clerck-Floate R, Ellison CA, Hinz HL, Hufbauer RA, Julien MH, Sforza R (2011) Applying molecular-based approaches to classical biological control of weeds. Biol Control 58:1–21

Gaskin JF, Wilson LM (2007) Phylogenetic relationships among native and naturalized *Hieracium* (Asteraceae) in Canada and the United States based on plastid DNA sequences. Syst Bot 32:478–485

Goolsby JA, De Barro PJ, Makinson JR, Pemberton RW, Hartley DM, Frohlich DR (2006a) Matching the origin of an invasive weed for selection of a herbivore haplotype for a biological control programme. Mol Ecol 15:287–297

Goolsby JA, van Klinken RD, Palmer WA (2006b) Maximising the contribution of native-range studies towards the identification and prioritisation of weed biocontrol agents. Austr J Entomol 45:276–286

Harms NE, Cronin JT, Diaz R, Winston RL (2020) A review of the causes and consequences of geographical variability in weed biological control successes. Biol Control 151:104398

Hillis DM, Moritz C, Mable BK (1996) Molecular Systematics. 2nd ed. Sunderland, MA: Sinauer Associates. 655 p

Hopkins AA, Taliaferro CM, Murphy CD, Christian D (1996) Chromosome number and nuclear DNA content of several switchgrass populations. Crop Science 36:1192–1195

Kaushal P, Paul S, Saxena S, Dwivedi KK, Chakraborti M, Radhakrishna A, Roy AK, Malaviya DR (2015) Generating higher ploidies (7x and 11x) in guinea grass (*Panicum maximum* Jacq.) utilizing reproductive diversity and uncoupled apomixis components. Curr Sci 109:1392–1395

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35:1547–1549

Maitland TD, Hubbard CE (1927) Notes on African grasses, V. Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew) 1927(7): 264–305

Mercadier G, Goolsby JA, Jones WA, Tamesse JL (2009) Results of preliminary survey in Cameroon, Central Africa, for potential natural enemies of *Panicum maximum* Jacq. (Poales: Poaceae), Guineagrass. Subtrop Plant Sci 61:31–36

Muir JP, Jank L (2004) Guineagrass. Pages 589–621 *in* Moser LE, Burson BL, Sollenberger LE, eds. Warm-Season (C4) Grasses. Agronomy Monograph No. 45. Madison, WI: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America

Nakajima K, Komatsu N, Mochizuki N, Suzuki S (1979) Isolation of diploid and tetraploid sexual plants in guineagrass (*Panicum maximum* Jacq.). Jpn J Breed 29:228–238

Rhodes AC, Plowes RM, Goolsby JA, Gaskin JF, Musyoka B, Calatayud PA, Cristofaro M, Grahmann ED, Martins DJ, Gilbert LE (2021) The dilemma of Guinea grass (*Megathyrsus maximus*): a valued pasture grass and a highly invasive species. Biol Invasions 23:3653–3669

Rohlf FJ (1992) NTSYS-PC: Numerical Taxonomy and Multivariate Analysis System. Setauket, NY: Exeter Software

Savidan Y, Pernès, J (1982) Diploid-tetraploid-dihaploid cycles and the evolution of *Panicum maximum* Jacq. Evolution 36:596–600

Soti P, Goolsby JA, Racelis AE (2020) Agricultural and environmental weeds of south Texas and their management. Subtropical Agric Environ 71:1–11

Sutton GF, Canavan K, Day MD, den Breeyen A, Goolsby JA, Cristofaro M, McConnachie A, Paterson ID (2019) Grasses as suitable targets for classical weed biological control. BioControl 64:605–622

Taberlet P, Geilly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17:1105–1109

Vacek AT, Goolsby JA, Calatayud PA, Le Ru B, Musyoka B, Kariyat RR (2021) Importation and preliminary evaluation of the stem boring moth *Buakea kaeuae* as a potential biological control agent of invasive Guineagrass, *Megathyrsus maximus*. Southwest Entomol 46:257–260

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M (1995) AFLP: a new technique for DNA-fingerprinting. Nucleic Acids Res 23:4407–4414

https://doi.org/10.1017/inp.2022.7 Published online by Cambridge University Press