

Human Settlement, Road and Rivers Rather than Climate Determine the Distribution of Giant Milkweed, *Calotropis procera* (Apocynaceae) in the Vhembe Biosphere Reserve, Limpopo Province, South Africa

¹Sipho Glen Mbambala, ¹Milingoni Peter Tshisikhawe, ²Peter John Taylor and ³Sebataolo Rahlao

¹Department of Botany, University of Venda, Private Bag X5050, Thohoyandou 0950, South Africa

²South African Research Chair on Biodiversity Value and Change, University of Venda, Private Bag X5050, Thohoyandou 0950, South Africa

³Biological Invasions Directorate, South African National Biodiversity Institute, Kirstenbosch National Botanical Gardens, Cape Town, South Africa

(Received 14 July, 2020; Accepted 22 April, 2021)

ABSTRACT

Alien plant invasion is one of the increasing threats to plant biodiversity and ecosystem services globally. Anthropogenic activities disturb ecosystems and cause a shift in community assemblages. In recent decades climate change has become the driving force in the disruption of ecosystems and the loss of species diversity, and in promoting species extinction and the spread of alien invasive plant species. In South Africa, 161 out of 8750 plant species are problematic and causing threats to biodiversity and 117 out of 161 alien invasive species are well-established and are widespread. Population counts of *Calotropis procera* were conducted for each kilometer consecutively for 20 km and 10 km on paved and unpaved road, respectively in the former Mutale Municipality of Vhembe District, Limpopo Province, South Africa. The existing GBIF (Global Biodiversity Information Facility) dataset of *Calotropis procera* historical collections from around the world was used to model the species distribution globally and in Southern Africa. Maxent 3.3.3k was used to model habitat suitability and the predicted current distribution of *Calotropis procera* using their distribution records after preparing and projecting to Albers equal area projection for the world. Four Bioclim climatic variables, Bio 1 (Annual Mean Temperature), Bio 4 (Temperature Seasonality), Bio 5 (Max Temperature of Warmest Month), and Bio 12 (Annual Precipitation) obtained from Worldclim database were used. QGIS 2.18.20, was used to create distribution maps of South Africa and Vhembe Biosphere Reserve (VBR) in relation to the position of roads, rivers and settlements. The total transformed areas (settlements), as well as roads and rivers, show new records of *Calotropis procera* fall in areas predicted to have low suitability. This study concludes that human settlements, urbanization, rivers and road construction influences the current distribution of *Calotropis procera* in the VBR

Key words: *Calotropis procera*, Maxent, Vhembe Biosphere Reserve.

Introduction

Biological invasion by alien invasive plant species is

one of the increasing threats to plant biodiversity and ecosystem services globally (Spooner, 2015; Jarosik *et al.*, 2011). Anthropogenic activities disturb

the ecosystems and cause a shift in community assemblages (Ammond and Litton, 2012). These disturbances contribute immensely to the colonisation and spread of alien invasive species (Rew and Johnson, 2010). *Calotropis procera* is native to tropical South Western Asia (Afghanistan, Pakistan, India, Iran, Arabia, Jordan), Africa (Egypt, Somalia, Libya, South Algeria, Morocco, Mauritania, Senegal), and has been introduced in the Caribbean, Central America, South America, Israel, Brazil and South Africa (Cavalcante *et al.*, 2016; Ibrahim *et al.*, 2015; Barbosa *et al.*, 2014; Leal *et al.*, 2013; Ibrahim *et al.*, 2012; De Freitas *et al.*, 2011; Brandes, 2005; Hanna *et al.*, 1999).

In the 21st century climate change is the driving force in the disruption of ecosystems and loss of species diversity, and in prompting species extinction and the distribution of alien invasive plant species (Mantyka-Pringle *et al.*, 2015; Pacifici *et al.*, 2015; Foden *et al.*, 2013). Land cover change due to climate change has therefore resulted in biodiversity loss (Visconti *et al.*, 2016; Jetz *et al.*, 2007), habitat loss and fragmentation (De Chazal and Rounsevell, 2009). However, 161 out of 8750 plant species are problematic and causing threats to biodiversity and 117 out of 161 alien invasive species are well-established and are widespread in South Africa (Semenya *et al.*, 2012).

Calotropis procera is a hairy lactiferous, evergreen, highly branched and perennial shrub belonging to the family Apocynaceae, well-known as Apple of Sodom, Swallow Wort or Giant Milk Weed. It is drought resilient, salt tolerant to a moderately great degree and grows to a height of about 3 - 5 m containing and producing large quantity of milky latex throughout the plant when injured. It is widespread in the tropics (Awaad *et al.*, 2018; Meena *et al.*, 2010; Junker *et al.*, 2009; Khan *et al.*, 2007). The stem is comprised of little primary stems developing from the soil and increasing growth to numerous secondary and tertiary stems. It is herbaceous above, tomentose, solid, cylindrical. The bark is soft and corky (Ranjan *et al.*, 2017; Singh *et al.*, 2017; Nascimento *et al.*, 2015). It is a harmful weed in grassland ecosystems, disturbed or overgrazed rangelands and poor managed fields (Heuzé *et al.*, 2016).

Calotropis procera has high seed production and high seed dispersal, and the dispersal mode is by wind, water, passing animals and vehicles, and the growth is fast when established in a favourable mi-

croclimatic and ecological conditions without human support (Leal *et al.*, 2013; Sobrinho *et al.*, 2013; Frosi *et al.*, 2012). Tropical rain from October to December assists the seeds to germinate and the growth is rapid in the wet season (Ranjan *et al.*, 2017). When the plant is about two years old the flowers develop between August to October, and the flowers stay open for 10 to 12 days (Ranjan *et al.*, 2017; Leal *et al.*, 2013). Between September to November, the fruits establish with many seeds, it then becomes ripe from November to February when the burst to release the seeds and the break of each season from October to December stimulates new growth and suckering (Ranjan *et al.*, 2017; Singh *et al.*, 2017; Barbosa *et al.*, 2014; Leal *et al.*, 2013; Frosi *et al.*, 2012).



Fig. 1. A matured *Calotropis procera* individual.

It is found and modified on sandy, acidic and high aluminum content soils, warm climate in dry soils, and has become widespread, appearing on grasslands and in the highlands. It has established as an exotic along roadsides, lagoon edges and in overgrazed native pastures. Its growth is flourishing on rubbish loads, waste or unplanted lands, along roadsides, sea shores the river banks. In India, it has received special consideration due to its use in traditional medicinal properties (Nascimento *et al.*, 2015;

Meena *et al.*, 2010; Juncker *et al.*, 2009; Ramos *et al.*, 2006).

The spread of alien invasive species is often facilitated by road verges, however this has not been tested, and an objective of this study was to relate *C. procera* distribution to the effect of roads. Meunier *et al.*, (2000), reported that mowing to manage road verges encourages a favourable habitat for small mammals in a landscape of intensive cropland. The effect of roads on wildlife is a relatively recent issue for road planners, builders, and managers to take into account at the planning stage (Munoz *et al.*, 2015). A rising concern about the ecological effects of roads on biodiversity has led to the rise of road ecology as a scientific discipline globally and in South Africa (Collinson *et al.*, 2015; Collinson *et al.*, 2014).

In semi-natural grasslands, some form of management is important to conserve high species richness because of the intense form of agricultural practice (Noordijk *et al.*, 2009). Hovd and Skogen (2005), reported that plant species structure and species numbers may differ significantly due to differences in ecological conditions and agricultural management. According to Cousins (2006), it is significant not only to assess the spatial effect on plant biodiversity at a local scale but also to include a historical effect, as it is well known that patterns of plant species distribution and abundance are influenced by landscape history.

Ecological niche modelling is designed to predict species occurrence at the current moment and also designed to understand why species occur at the particular area. Environmental variables and known species distribution records are utilized to model the current occurrence of a species and also to detect the environmental conditions in which the population can be conserved. The environment spatial occurrence and the habitat suitability of the species can be projected throughout the study area (Elith and Leathwick 2009; Peterson and Nakazawa 2008; Ficetola *et al.*, 2007; Pearson 2007).

Alien invasive plant species such as *C. procera* act as corridors by the roadside, and the effects of opening paths in the form of roads for vehicles, human movement and human settlements tend to spread of alien invasive plant species (Street and Prinsloo, 2013). Rahlao *et al.*, (2010) noted that the establishment of alien invasive plant species is actively promoted by the disturbed conditions at the roadsides. In the Vhembe Municipality, there is an escalating

number of alien invasive plant species which are out-competing native species (Mbambala *et al.*, 2017). Little research studies has been conducted in the former Mutale Local Municipality regarding the future distribution of *C. procera*, specifically on roadside verges.

The aim of the study was to examine the current and possible modelled distribution with regards to the impact of *C. procera* in Vhembe Biosphere Reserve (VBR) in Limpopo Province in South Africa. Climate factors can also determine the spread of alien invasive species, as can flooding of rivers (Taylor *et al.*, 2012). The study aimed to test the relative influence of environment (climate and rivers) versus human (roads and settlements) factors on the distribution of *Calotropis procera*.

Materials and Methods

Study area

The population study was conducted in the former Mutale Local Municipality (1,500 m²), which was one of the four local municipalities making up Vhembe District Municipality (VBR). It is situated in the far north eastern corner of the district (Stats SA, 2011). The Kruger National Park forms the eastern boundary of Mutale Local Municipality, with the Limpopo River forming the north eastern boundary. The Municipality's strategic location affords it many opportunities for tourism development. The local municipality has a total population of 131 215 (Stats SA, 2011). It is estimated that 24 239 households live in the municipality area with an average household size of five persons (Stats SA, 2011). This area has since been incorporated into the Musina Local Municipality.

Mutale Local Municipality shared its borders with Musina Local Municipality and Zimbabwe on the North, Mozambique to the East, Makhado Local Municipality to the west and Thulamela Local Municipality to the South (Stats SA, 2011). The municipality was accessed through R525 paved road linking the Kruger National Park to the other local municipalities within Vhembe District (Stats SA, 2011).

Mutale Local Municipality as a rural municipality had a lot of opportunities for development. According to Statistics South Africa (2011), opportunities that can speed up development have been identified in area of agriculture, mining, tourism, arts and culture.

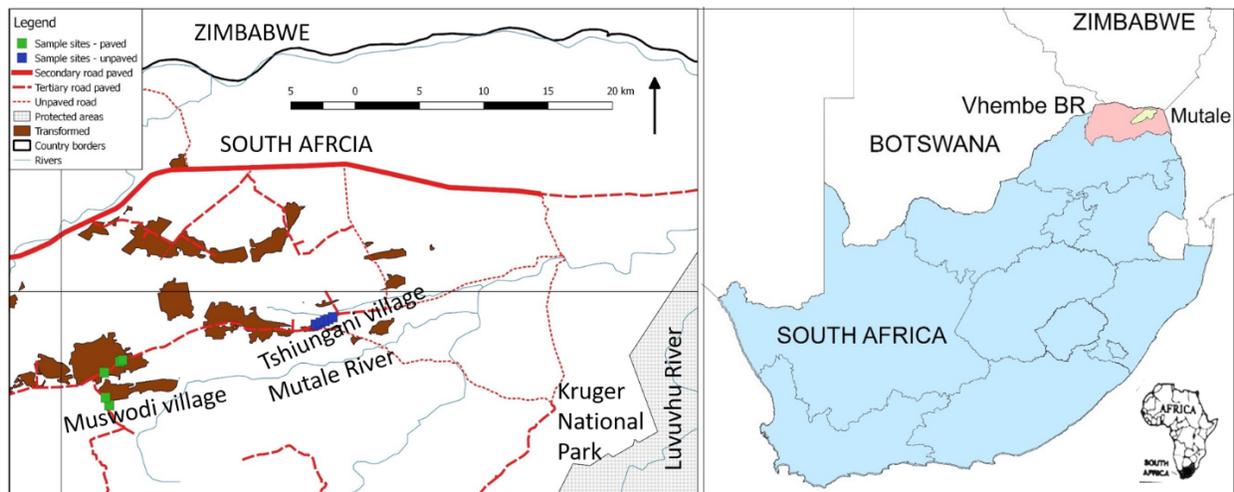


Fig. 2. A Locality map showing the study area, whereas population counts were obtained for *Calotopis procera* along two transects of paved and unpaved roads in former Mutale Local Municipality, located within the VBR, Limpopo Province, South Africa.

The Maxent study obtained projections of habitat suitability for *C. procera* for the VBR region based on a global model. In addition to records obtained for the former Mutale Local Municipality, existing public records Sapia (South African Plant Invaders Atlas: www.arc.agric.za/arc-ppri/NewsArticlesLibrary/Henderson.pdf), GBIF (Global Biodiversity Information Facility: <http://www.gbif.org/species/5414566>) and Cath Vise, a PhD Candidate at the University of Venda, were plotted on a map of the VBR overlain with habitat suitability, human settlements, road and river networks, to better comprehend the importance of climate predictions versus disturbances factors in the current spread of *C. procera* in the VBR.

Methodology

Population counts

A single observer used the drive-by survey technique (as explained by Dean and Milton, 1998) that made use of overland long distance journeys to record the presence and absence and number of individuals of invasive alien species at 1 km intervals. Sites were selected using the drive-by survey (as explained by Rahlao *et al.*, 2010). A belt transect (a continuous belt similar to a line transect but it provides information on the presence and absence of plant species in addition to the abundance) was used for every 1 km. The density estimates for *C.*

procera were counted along the roadside in a moving vehicle at the speed of 40km/h (Sharma *et al.*, 2010). Population counts were conducted consecutively every 1km for 20 km and 10 km on the paved road and unpaved road, respectively. PAST (PAleontologicalSTatistics) 3.20 (downloaded from <https://palaeo-electronica.org/2001> on 21 August 2018), was used to do a bivariate regression of *C. procera* counts and distance from nearest settlements.

Species distribution modeling

The use of the existing GBIF (Global Biodiversity Information Facility) datasets (<http://www.gbif.org/species/5414566>) of *C. procera* were used to model the species distribution globally and in Southern Africa. QGIS 2.18.20 (downloaded from <https://www.qgis.org> on 21 August 2018) was used to generate species distribution map for all *C. procera* found globally. Maxent 3.3.3k (Phillips *et al.*, 2006) was used to model current spread of *C. procera* using their distribution records after preparing and projecting to Albers equal area projection for the world. QGIS 2.18.20 was used to create distribution maps of South Africa and Vhembe Biosphere Reserve. The natural distribution records were used to make a simple model of suitable habitats in the VBR (Taylor *et al.*, 2012). This helped in comparing the potential spread in the VBR and identification of priority areas for control at a Biosphere level.

When running Maxent, standard defaults were used, present records were split, 70% training and 30% testing. Five replicates were run using cross validation. The background was selected using a mask for the plant species based on polygon of all occurrence from points within a 200 km buffer was added in order to prevent problems of over-fitting or under-fitting. Three threshold were used, Fixed cumulative value 10 (low suitability) at 0.261, 10 percentile training presence (intermediate suitability) at 0.346 and Equal training sensitivity and specificity (suitable) at 0.456, was used to display maps of prediction from continuous probability data and to define categories such that they can relate to suitability of the plant species. Four Bioclim climatic variables, Bio 1 (Annual Mean Temperature), Bio 4 (Temperature Seasonality), Bio 5 (Max Temperature of Warmest Month), and Bio 12 (Annual Precipitation) were obtained from Worldclim database (www.worldclim.org; Peterson and Nakazawa 2008).

Since it seems challenging to exactly differentiate native from introduced records, the Maxent model was based on total range (indigenous and introduced) records. Generally for alien invasive plant species, one uses only native range to make the model and then project the model onto the potential invaded areas. In this study, we wanted to validate combining native and non-native records due to the difficulty in differentiating exactly the native range.

In addition, we were interested in the habitat suitability (based on model probabilities) in the VBR and Africa more generally, where we can be sure that for Southern and Eastern Africa at least this was not part of the original native range. Therefore, the approach of the study was to project the world habitat suitability (climatic) model onto the VBR region and to overlay the recent occurrence records (from Cath Vise a PhD Candidate at University of Venda, SAPIA, and road transects survey). However, these new records were not used to build the model, only historical GBIF records were used for the global model. Hence they can be used as a test of how important the climate model is in explaining the spread of the species in South Africa/VBR.

Results

Figure 3 shows the *C. procera* records distribution from other sources, i.e. Southern African Plant Invaders Atlas (SAPIA), GBIF, Cath Vise and road transect survey. The map shows that *C. procera* is distributed in transformed settlements.

Figure 4 shows the 30 sites in which a road count survey for *C. procera* was conducted. It is clear that there is a greater number of *C. procera* individuals counted in and close to settlements due to the human disturbances activities conducted. There is no difference in abundance of *C. procera* along paved compared to unpaved roads. Although a higher

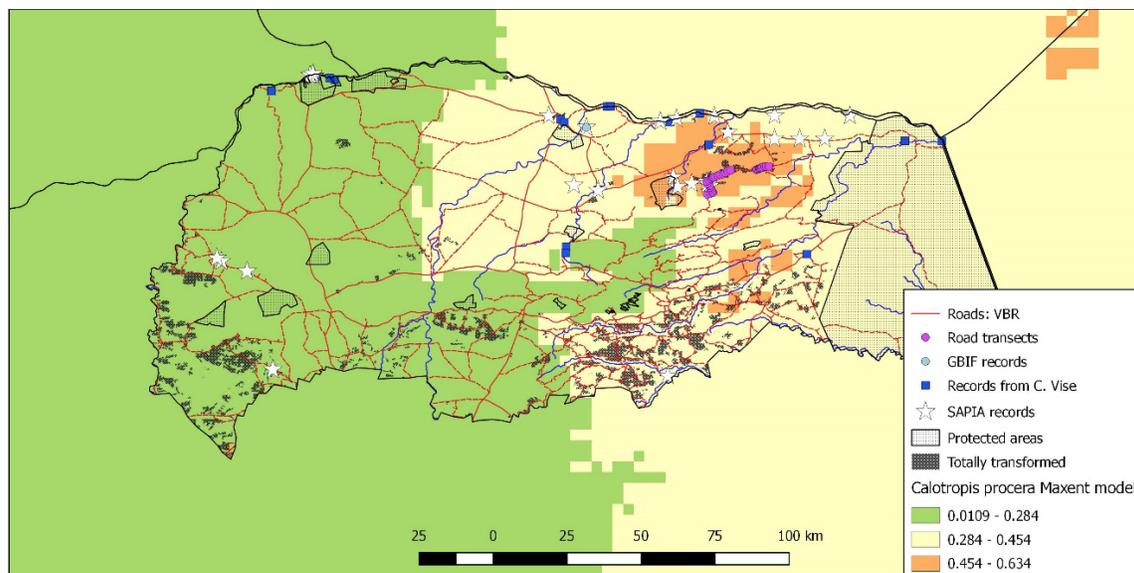


Fig. 3. Different distribution records of *Calotropis procera* from different sources in VBR overlain with Maxent probabilities.

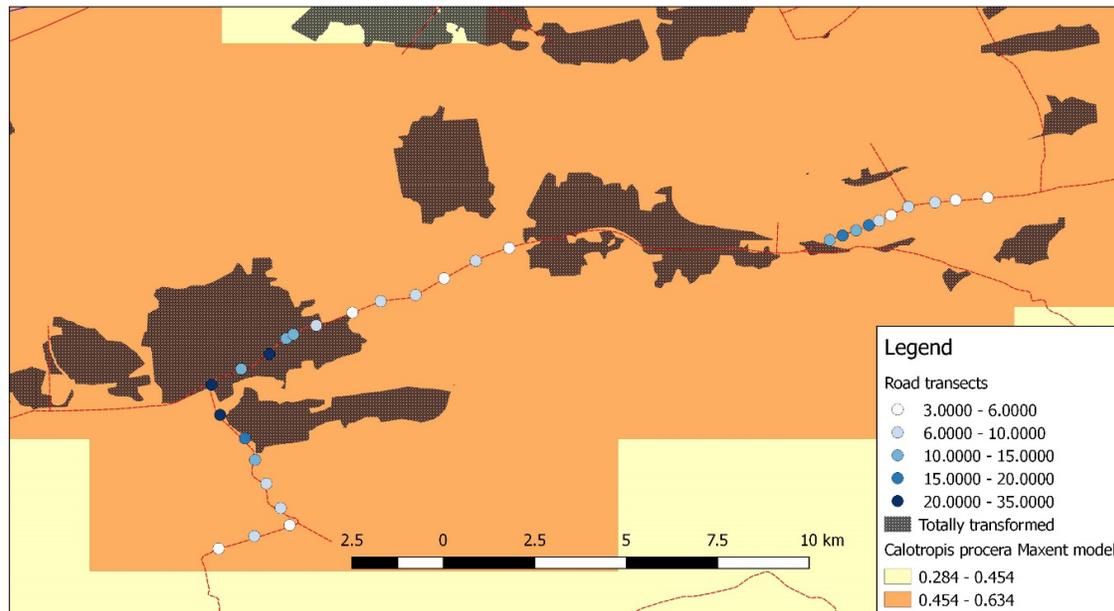


Fig. 4. A map showing a road transect survey of population counts of *Calotropis procera* conducted every 1km. The transect on the left represent a paved road and that on the right an unpaved road. Colour indicates number of individuals as per legend.

maximum population size of 20-23 individuals was counted along the paved road, this section of the road formed part of a much larger settlement than was the case for the unpaved road. Outside the settlements, low population sizes of 3-10 individual were found along both paved and unpaved roads.

Figure 5 shows a significantly negative regression *C. procera* abundance and settlements. Distance explained 40% of variation in density estimates, $p < 0.001$.

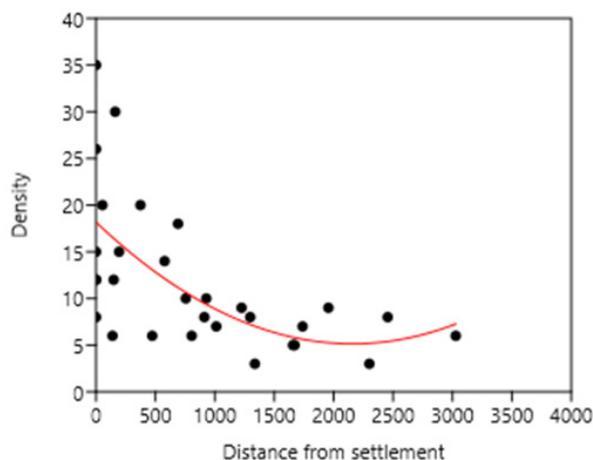


Fig. 5. Second order polynomial regression of *Calotropis procera* density versus distance from settlements (in meters) from road transect surveys.

Maxent results

The model performed well with Area Under the Curve (AUC) values of 0.775 for both the training and test datasets, which specifies the fit of the model to the testing data.

Figure 6 shows the global scale distribution of *C. procera* in continents such as Australia, South America, Africa and Asia. It is native to tropical South Western Asia (Pakistan, Afghanistan, India, Iran, Arabia, Jordan), and has been introduced in Africa (Egypt, Somalia, Libya, south Algeria, Morocco, Mauritania, Senegal), Caribbean, Central America, South America, Israel, Brazil and South Africa (Cavalcantea *et al.*, 2016; Ibrahim *et al.*, 2015; Barbosa *et al.*, 2014; Leal *et al.*, 2013; Ibrahim *et al.*, 2012; De Freitas *et al.*, 2011; Brandes, 2005; Hanna *et al.*, 1999).

The area showing the highest probability of occurrence of *C. procera* (red shading) are West Africa (Mali, Ghana, Ivory Coast, Guinea and Mauritania), Central Africa (Sudan, Equatorial Guinea and Gabon), East Africa (Somalia) and Southern Africa (Madagascar). The occurrence probability is moderate or less in areas shaded in green and yellow.

Figure 3 shows that the probabilities of *C. procera* occurrence in VBR. The probability of occurrence is therefore highest towards the northern part of VBR.

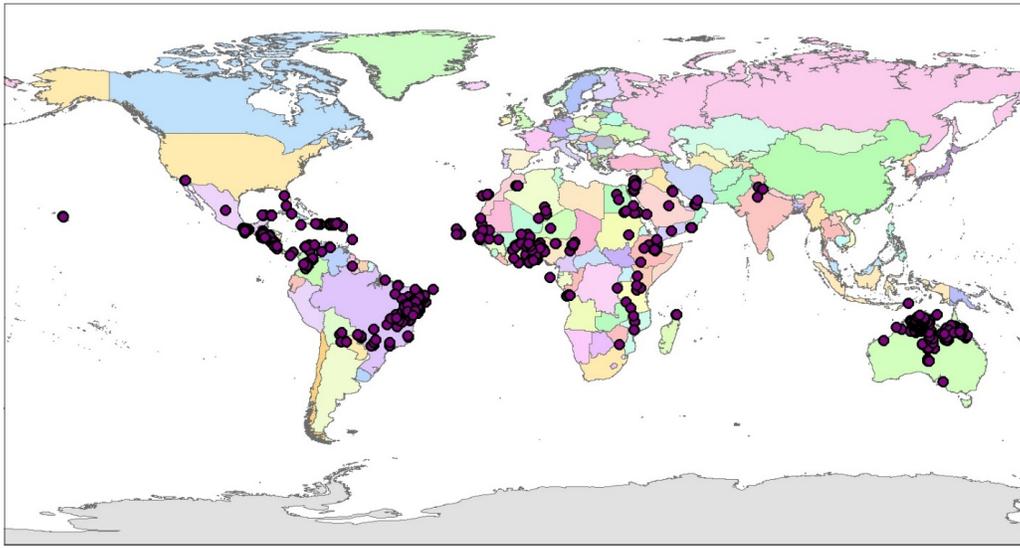


Fig. 6. Global distribution of *Calotropis procera*.

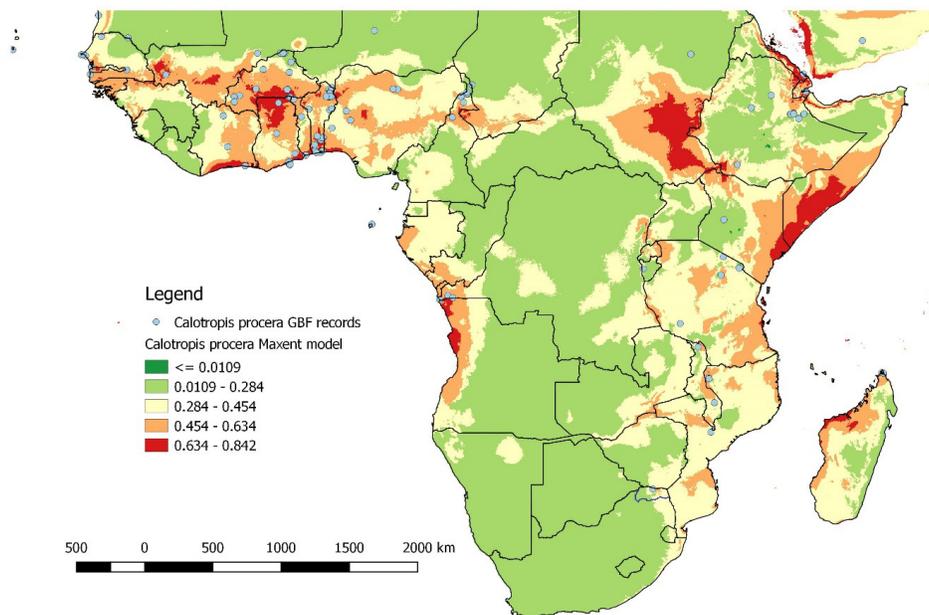


Fig. 7. Showing Maxent probabilities overlaid by distribution records of *Calotropis procera* GBIF records in Africa.

Figure 8 shows that the habitat suitability is low when the Mean Annual Temperature is between 0 °C and 19 °C and the suitability increases as temperature increases and habitat suitability of *C. procera* is 29 °C the species prefer to grow under high temperature. Habitat suitability decreases as the temperature increase to 30 °C and beyond.

Figure 9 shows that the population is low when the temperature seasonality is low at 0 °C and the population decreases the temperature is around 5

°C. The population increases as temperature increases and habitat suitability of *C. procera* is 60 °C which is the temperature seasonality, the species prefers to grow under extremely hot temperature.

Figure 10 shows that the population of the species will increase when the maximum temperature of the warmest month is around 26-29 °C and habitat suitability of *C. procera* is 29 °C. The population decreases as the temperature increase to 30 °C and beyond.

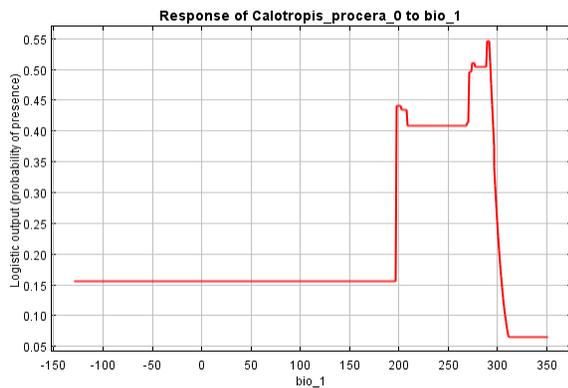


Fig. 8. Maxent model prediction for Bio 1 (Annual Mean Temperature).

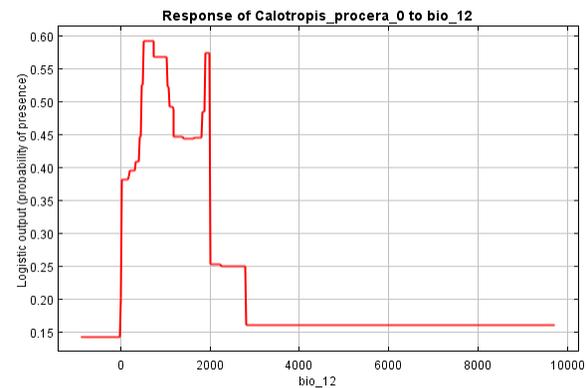


Fig. 11. Maxent model prediction for Bio 12 (Annual Precipitation).

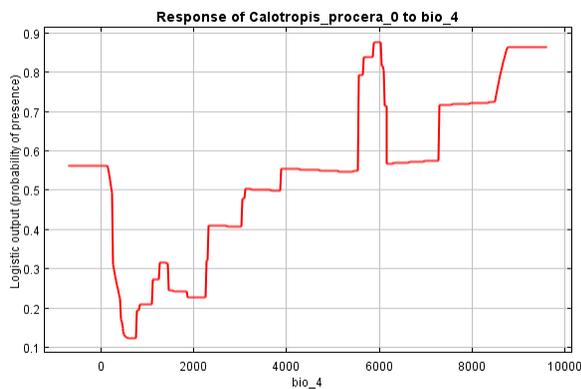


Fig. 9. Maxent model prediction for Bio 4 (Temperature Seasonality).

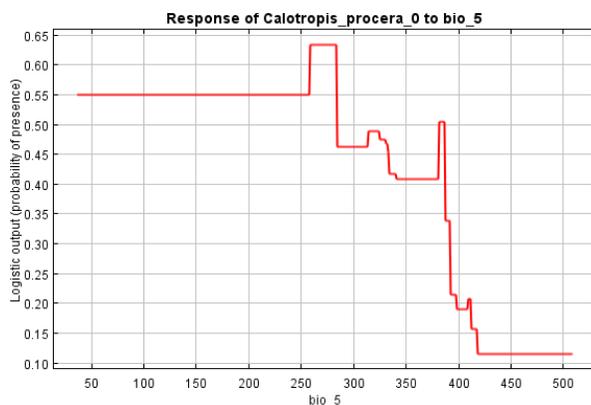


Fig. 10. Maxent model prediction for Bio 5 (Maximum Temperature of Warmest Month).

Figure 11 shows that the habitat suitability of *Calotropis procera* is highest between about 100 mm and 2000 mm precipitation. The population decreases as the precipitation increase from 2100 mm and beyond.

In table 1, Bio 1 has the highest percent contribution, the environmental variable with maximum gain when used in isolation is Bio 1, hence it appears to have the most useful information by itself. The environmental variable that decreases the gain the most when it is omitted is Bio 4, hence it appears to have the most information that isn't present in the other variables. The four final variables used in our model (Bio1, 4, 5 and 12) are largely un-correlated with each when compared to all 19 Bioclim variables (Fig. 13), justifying their selection as representing independent ecological dimensions

Discussion and Conclusion

MAXENT is an effective tool in predicting the current occurrence of many species and how they respond to the changes in climatic conditions (Taylor *et al.*, 2017; 2016; Fandohan *et al.*, 2015), however, in this study MAXENT did not produce good results in predicting species presence because only climatic variable were used in predicting the current distribution of *C. procera*. In addition, the study reveals that roads, rivers and settlements/disturbances contribute to the current distribution of *C. procera*. In studies conducted by Schoeman *et al.*, (2013); Ficetola *et al.*, (2007); Pearson (2007), it was reported that using modelling techniques in the prediction of occurrence of species by linking distribution records with digital layers of environmental variables is very greatly important in conservation practice and for the risk assessment.

Human activities often facilitate the introduction alien invasive species in an area. In studies conducted by Wan *et al.*, (2017); Dellinger *et al.*, (2016);

Table 1. Estimates of relative contributions of the environmental variables to the Maxent model.

| Variable | Percent contribution | Permutation importance |
|--|----------------------|------------------------|
| Bio 1 (Annual Mean Temperature) | 29.8 | 5.6 |
| Bio 4 (Temperature Seasonality) | 19.3 | 24.2 |
| Bio 5 (Max Temperature of Warmest Month) | 16.1 | 12.8 |
| Bio 12 (Annual Precipitation) | 15.6 | 19.2 |

Fandohan *et al.*, (2015); Follak and Strauss, (2010), it was reported that human activities such as transportation, agricultural practice and trade are amongst the factors that contribute to the formation of alien invasive plant species in an area.

The total transformed areas (settlements), as well as roads and rivers, show new records of *C. procera* fall in areas predicted to have low suitability. These isolated records (in the west of VBR, Mapungubwe, Pafuri in Kruger) seem to be explained by distribution of roads, settlements and rivers, hence human disturbance as well as flooding and spreads of seeds along Limpopo and Levubu River. These results are related to those of Ficetola *et al.*, (2007), showed that human activities greatly increase species invasion and distribution.

In the study conducted by Taylor *et al.*, (2012), it was reported that cold stress can be a limiting factor for distribution the invasive of *Lantana camara*. The current study identified future areas that may be at threat of *C. procera* invasion due to climate change. These are areas such as Kruger National Park, Thohoyandou, Giyani, Musina, Mpumalanga, Louis Trichardt and Polokwane. The study also identified that factors such as human settlements and disturbances as possibly influence current distribution of *C. procera*.

According to Sharma *et al.*, (2010), it was reported

that the spread zonation of alien invasive plants ranging from rural to urban area due to urbanization. Jamie *et al.*, (2017) showed that bulldozing which is also a way of paving roads alters the ecosystems, hence promoting the alien invasive species spread. Since alien invasive plant species promote biodiversity loss and reduction in ecosystem ser-

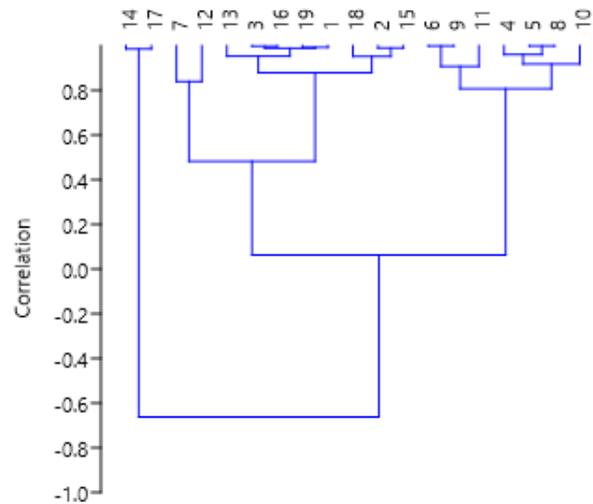


Fig. 13. Correlation Unweighted Pair Group Method with Arithmetic Mean (UPGMA) phenogram showing relationships among 19 Bioclim variables for all *Calotropis procera* records (world-wide).

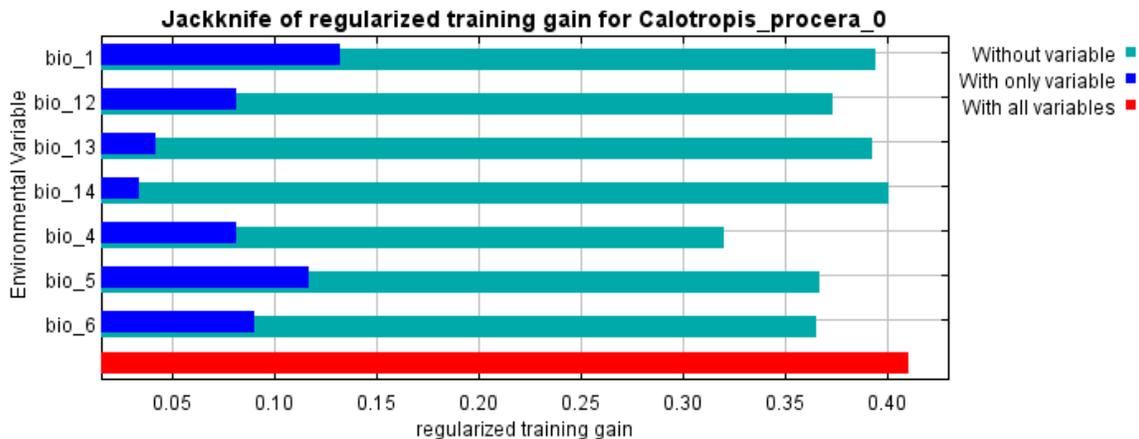


Fig. 12. Maxent model results of the jackknife test of variable importance.

vices (Dalmazzone and Giaccaria, 2014; Gooden and French, 2014), this implies that road ecology also has an impact in driving biodiversity loss and associated ecosystem services.

This study concludes MAXENT is still an important tool in predicting the current occurrence since it shows where the species occur as a result of climatic niche. However, in any case where records do not correspond they can reveal other factors such as disturbances. The study also concludes that human settlements have a large effect as several SAPIA records fall in the areas of Western VBR. Climatic predictions indicate low suitability of *C. procera*, yet the proximity of records with road networks and human settlements indicate that these factors probably coincided with their accidental introduction and spread. Records along the Limpopo River indicate that rivers and flooding disturbances can also result in accidental propagation of seeds of invasive species like *C. procera* even in areas which are not climatically suitable.

So all these factors (human settlements, urbanization, road construction and flooding of rivers) have influenced the current distribution of *Calotropis procera* in Northern Limpopo and will probably play an increasing role in its spread unless management action can be taken. The study recommends variables related to human disturbances, roads and rivers could try to be included in the model to improve the predictive performance for planning where to prioritize conservation control and practice.

Acknowledgements

The authors are grateful to the University of Venda's Research and Publication Committee for their financial support. Project Number SMNS/17/BOT/06

Reference

- Ammond, S.A. and Litton, C.M. 2012. Competition between native Hawaiian plants and the invasive grass *Megathyrsus maximus*: implications of functional diversity for ecological restoration. *Restoration Ecology*. 20 : 638-646.
- Awaad, A.A., Alkanhal, H.F., El-Meligy, R.M., Zain, G.M., Adri, V.D.S., Hassan, D.A. and Alqasoumi, S.I. 2018. Anti-ulcerative colitis activity of *Calotropis procera* linn. *Saudi Pharmaceutical Journal*. 26(1): 75-78.
- Barbosa, M.O., De Almeida-Cortez, J.S., Da Silva, S.I. and De Oliveira, A.F. 2014. Seed oil content and fatty acid composition from different populations of *Calotropis procera* (Aiton) W. T. Aiton (Apocynaceae). *Journal of the American Oil Chemists' Society*. 91 : 1433-1441.
- Brandes, D. 2005. *Calotropis procera* on Fuerteventura. *Technical University Braunschweig, Germany*. 1-7.
- Cavalcante, G.S., De Moraes, S.M., Andre, W.P.P., Ribeiro, W.L.C., Rodrigues, A.L.M., De Lira, F.C.M.L., Viana, J.M. and Bevilacqua, C.M.L. 2016. Chemical composition and in vitro activity of *Calotropis procera* (Ait.) latex on *Haemonchus contortus*. *Veterinary Parasitology*. 226: 22-25.
- Collinson, W.J., Parker, D.M., Bernard, R.T.F., Brian K. Reilly, B.K. and Davies-Mostert, H.T. 2014. Wildlife road traffic accidents: a standardized protocol for counting flattened fauna. *Ecology and Evolution*. 4(15): 3060-3071.
- Collinson, W.J., Parker, D.M., Bernard, R.T.F., Brian K. Reilly, B.K. and Davies-Mostert, H.T. 2015. An inventory of vertebrate roadkill in the Greater Mapungubwe Transfrontier Conservation Area, South Africa. *African Journal of Wildlife Research*. 45(3): 301-311.
- Cousins, S.A.O. 2006. Plant species richness in midfield islets and road verges- The effect of landscape fragmentation. *Biological Conservation*. 127: 500-509.
- Dalmazzone, S. and Giaccaria, S. 2014. Economic drivers of biological invasions: A worldwide bio-geographic analysis. *Ecological Economics*. 105: 154-165.
- De Chazal, J. and Rounsevell, M.D.A. 2009. Land-use and climate change within assessments of biodiversity change: a review. *Global Environmental Change-Human Policy Dimension*. 19 : 306-315.
- De Freitas, C.D.T., Nogueira, F.C.S., Vasconcelos, I.M., Oliveira, J.T., Domont, G.B. and Ramos, M.V. 2011. Osmotin purified from the latex of *Calotropis procera*: Biochemical characterization, biological activity and role in plant defense. *Plant Physiology and Biochemistry*. 49 : 738-743.
- Dean, W.R.J. and Milton, S.J. 1998. Alien plant assemblages near roads in arid and semi-arid South Africa. *A Journal of Conservation Biogeography*. 4(4): 175-187.
- Dellinger, A.S., Essl, F., Hojsgaard, D., Kirchheimer, B., Klatt, S., Dawson, W., Pergl, J., Pysek, P., van Kleunen, M., Weber, E. and Winter, M. 2016. Niche dynamics of alien species do not differ among sexual and apomictic flowering plants. *New Phytologist*. 209(3): 1313-1323.
- Elith, J. and Leathwick, J.R. 2009. Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution and Systematics*. 40 : 677-97.
- Fandohan, A.B., Oduor, A.M., Sodé, A.I., Wu, L., Cuni-Sanchez, A., Assédé, E. and Gouwakinnou, G.N. 2015. Modeling vulnerability of protected areas to invasion by *Chromolaena odorata* under current and future climates. *Ecosystem Health and Sustainability*.

- 1(6) : 1-12.
- Ficetola, G.F., Thuiller, W. and Miaud, C. 2007. Prediction and validation of the potential global distribution of a problematic alien invasive species—the American bullfrog. *Diversity and Distributions*. 13(4) : 476-485.
- Foden, W.B., Butchart, S.H., Stuart, S.N., Vié, J.C., Akçakaya, H.R., Angulo, A., DeVantier, L.M., Gutsche, A., Turak, E., Cao, L. and Donner, S.D. 2013. Identifying the world's most climate change vulnerable species: a systematic trait-based assessment of all birds, amphibians and corals. *PloSone*. 8(6) : e65427.
- Follak, S. and Strauss, G. 2010. Potential distribution and management of the invasive weed *Solanum carolinense* in Central Europe. *Weed Research*. 50(6) : 544-552.
- Frosi, G., Oliveira, M.T., Almeida-Cortez, J. and Santos, M.G. 2012. Ecophysiological performance of *Calotropis procera*: an exotic and evergreen species in Caatinga, Brazilian semi-arid. *Acta Physiologia Plantarum* 35: 335-344.
- Gooden, B. and French, K. 2014. Impacts of alien grass invasion in coastal seed banks vary amongst native growth forms and dispersal strategies. *Biological Conservation*. 171 : 14-126.
- Hanna, A.G., Elgamal, M.H.A., Morsy, N.A.M., Duddeck, H., Kovács, J. and Tóth, G. 1999. Two cardenolides from *Calotropis procera*. *Magnetic Resonance in Chemistry*. 37 : 754-757.
- Heuzé, V., Tran, G., Baumont, R. and Bastianelli, D. 2016. *Calotropis (Calotropis procera)*. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. [ONLINE] Available at: <https://www.feedipedia.org/node/588>. [Accessed 3 September 2018].
- Hovd, H. and Skogen, A. 2005. Plant species in arable field margins and road verges of central Norway. *Agriculture, Ecosystems and Environment*. 110 : 257-265.
- Ibrahim, S.R.M., Mohamed, G.A., Shaala, L.A., Banuls, L.M., Kiss, R., D. and Youssef, D.T.A. 2015. Calotroposides H-N, new cytotoxic oxypregnaneoligoglycosides from the root bark of *Calotropis procera*. *Steroids*. 96 : 63-72.
- Jamie, X.A., VamBloem S.J., Koch, F.H. and Nelson, S.A.C. 2017. Spread of common native and invasive grasses and ruderal trees following anthropogenic disturbances in a tropical dry forest. *Ecological Processes*. 6(38): 1-14.
- Jarosik, V., Pysek, P., Foxcroft, L.C., Richardson, D.M., Rouget, M. and MacFadyen, S. 2011. Predicting Incursion of Plant Invaders into Kruger National Park, South Africa: The Interplay of General Drivers and Species-Specific Factors. *PLoSone*. (6)12: e28711. doi:10.1371/journal.pone.0028711.
- Jetz, W., Wilcove, D.S. and Dobson, A.P. 2007. Projected impacts of climate and land-use change on the global diversity of birds. *PLoS biology*. 5(6) : p.e157.
- Juncker, T., Schumacher, M., Dicato, M. and Diederich, M. 2009. UNBS1450 from *Calotropis procera* as a regulator of signaling pathways involved in proliferation and cell death. *Biochemical Pharmacology*. 78 : 1-10.
- Khan, R., Shahzad, S., Choudhary, M.I., Khan, S.A. and Ahmad, A. 2007. Biodiversity of the endophytic fungi isolated from *Calotropis procera* (Ait.) R. Br. *Pakistan Journal of Botany*. 39(6): 2233-2239.
- Leal, L.C., Meiado, M.V., Lopes, A.V. and Leal, I.R. 2013. Germination responses of the invasive *Calotropis procera* (Ait.) R. Br. (Apocynaceae): comparisons with seeds from two ecosystems in northeastern Brazil. *Annals of the Brazilian Academy of Sciences*. 85(3): 1025-1034.
- Mantyka-Pringle, C.S., Visconti, P., Di Marco, M., Martin, T.G., Rondinini, C. and Rhodes, J.R. 2015. Climate change modifies risk of global biodiversity loss due to land-cover change. *Biological Conservation*. 187: 103-111.
- Mbambala, S.G., Tshisikhawe, M.P. and Masevhe, N.A. 2017. Invasive alien plants used in the treatment of HIV/AIDS-related symptoms by traditional healers of Vhembe municipality, Limpopo Province, South Africa. *African Journal of Traditional, Complementary and Alternative Medicines*. 14(5) : 80-88.
- Meena, K.A., Yadav, K.A., Panda, P., Preet, K. and Rao, M.M. 2010. Review on *Stereospermum suaveolens* DC: A Potential Herb. *Drug Invention Today*. 2(5): 238-239.
- Meunier, F.D., Verheyden, C. and Jouventin, P. 2000. Use of roadsides by diurnal raptors in agricultural landscapes. *Biological Conservation*. 92 : 291-298.
- Munoz, P.T., Torres, F.P. and Megias, A.G. 2015. Effects of roads on insects: a review. *Biodiversity and Conservation* 24 : 659-682.
- Nascimento, T.L., Oki, Y., Lima, D.M.M., Almeida-Cortez, J.S., Fernandes, G.W. and Souza-Motta, C.M. 2015. Biodiversity of endophytic fungi in different leaf ages of *Calotropis procera* and their antimicrobial activity. *Fungal Ecology*. 14 : 9-86.
- Noordijk, J., Delille, K., Schaffers, A.P. and Sykora, K.V. 2009. Optimizing grassland management for flower-visiting insects in roadside verges. *Biological Conservation*. 142 : 2097-2103.
- Pacifici, M., Foden, W.B., Visconti, P., Watson, J.E., Butchart, S.H., Kovacs, K.M., Scheffers, B.R., Hole, D.G., Martin, T.G. and Akçakaya, H.R. 2015. Assessing species vulnerability to climate change. *Natural Climate Change*. 5 : 215-224.
- Pearson, R.G. 2007. Species' Distribution Modeling for Conservation Educators and Practitioners. Synthesis. American Museum of Natural History. Available at <http://ncep.amnh.org>.
- Peterson, A.T. and Nakazawa, Y. 2008. Environmental data sets matter in ecological niche modelling: an

- example with *Solenopsis invicta* and *Solenopsis richteri*. *Global Ecology and Biogeography*. 17(1): 135-144.
- Phillips, S.J., Anderson, R.P. and Schapire, R.E. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling*. 190(3-4): 231-259.
- Rahlao, S.J., Milton, S.J., Esler, K.J. and Barnard, P. 2010. The distribution of invasive *Pennisetum etaceum* along roadsides in western South Africa: the role of corridor interchanges. *Weed Research*. 10 : 1365-3180.
- Ramos, M.V., Bandeira, G.P, De Freitas, C.D., Nogueira, N.A., Alencar, N.M., De Sousa, P.A. and Carvalho, A.F. 2006. Latex constituents from *C. procera* (R. Br.) display toxicity upon egg hatching and larvae of *Aedes aegypti* (Linn.). *Memórias do Instituto Oswaldo Cruz*. 101(5) : 503-510.
- Ranjan, N., Singh, S.K. and Kumari, C. 2017. Biological Morphology and Ethano-Pharmacological Importance of *Calotropis* Species-A Review. *International Journal of Current Microbiology and Applied Sciences*. 6(4): 1640-1648.
- Rew, L.J. and Johnson, M.P. 2010. Reviewing the role of wildfire on the occurrence and spread of invasive plant species in wildland areas of the intermountain western United States. *Invasive Plant Science and Management*. 3(4): 347-364.
- Schoeman, M.C., Cotterill, F.P.D., Taylor, P.J. and Monadjem, A. 2013. Using potential distributions to explore environmental correlates of bat species richness in southern Africa: effects of model selection and taxonomy. *Current Zoology*. 59(3) : 1-15.
- Semenya, S.S., Tshikhawe, M.P. and Potgieter, M.T. 2012. Invasive alien plant species: A case study of their use in the Thulamela Local Municipality, Limpopo Province, South Africa. *Scientific Research and Essays*. 7(27) : 2363-2369.
- Sharma, G.P., Kumar, M. and Raghubanshi, A.S. 2010. Urbanization and road-use determines *Calotropis procera* distribution in the eastern Indo-Gangetic plain, India. *Ambio*. 39 (2) : 194-197.
- Singh, A.K., Dubey, S.N. and Sahu, R.K. 2017. On the morphology and taxonomy of two multipurpose congeneric taxa *Calotropis gigantea* and *C. procera* (Apocynaceae). *Asian Journal of Science and Technology*. 8(09): 5774-5782.
- Sobrinho, M.S., Tabatinga, G.M., Machado, I.C. and Lopes, A.V. 2013. Reproductive phenological pattern of *Calotropis procera* (Apocynaceae), an invasive species in Brazil: Annual in native areas; continuous in invaded areas of Caatinga. *Acta Botanica Brasilica*. 27 : 456-459.
- Spooner, P.G. 2015. Minor rural road networks: values, challenges, and opportunities for biodiversity conservation. In: Seiler A, Helldin J-O (Eds) Proceedings of IENE 2014 International Conference on Ecology and Transportation, Malmö, Sweden. *Nature Conservation*. 11 : 129-142.
- Statistics South Africa. 2011. *Community Survey, 2007*. Pretoria: Statistics South Africa 2011.
- Street, R.A. and Prinsloo, G. 2013. Commercially Important Medicinal Plants of South Africa: A Review. *Journal of Chemistry*. 1-16.
- Taylor, P.J., Nengovhela, A., Linden, J. and Baxter, R.M. 2016. Past, present, and future distribution of Afromontane rodents (Muridae: Otomys) reflect climate-change predicted biome changes. *Mammalia*. 80(4) : 359-375.
- Taylor, P.J., Ogony, L., Ogola, J. and Baxter, R.M. 2017. South African mouse shrews (*Myosorex*) feel the heat: using species distribution models (SDMs) and IUCN Red List criteria to flag extinction risks due to climate change. *Mammal Research*. 62 (2) : 149-162.
- Taylor, S., Kumar, L., Reid, N. and Kriticos, D.J. 2012. Climate change and the potential distribution of an invasive shrub, *Lantana camara* L. *PloS One*. 7(4) : p.e35565.
- Visconti, P., Bakkenes, M., Baisero, D., Brooks, T., Butchart, S.H., Joppa, L., Alkemade, R., Marco, M.D., Santini, L. and Hoffmann, M. 2016. Projecting global biodiversity indicators under future development scenarios. *Conservation Letters*. 9(1): 5-13.
- Wan, J.Z., Wang, C.J., Tan, J.F. and Yu, F.H. 2017. Climatic niche divergence and habitat suitability of eight alien invasive weeds in China under climate change. *Ecology and Evolution*. 7(5): 1541-1552.
-