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Differences in germination traits of time-separated seed collections of dry forest species from Central Zambia and the potential role of climate

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Abstract

Time-separated seed collections with a separation period of 12–18 years were used to study recent changes in germination traits and the role of climate factors in seven tropical dry forest (TDF) woody species in Zambia, southern Africa. In all the species, peak and final germination were lower in recent (2016–2021) seed collections than in historic (1998–2005) seed collections during the first germination season. However, in species with seed dormancy, both peak and final germination after one year burial were higher in recent seed collections than in historic seed collections. Three monthly climate factors had significant effects on final germination in the different species and the timing of most of these factors was during seed development and ripening that suggest that these factors operated through mother plants. In species with seed dormancy, post-first-year germination of buried seeds responded to climate factors in the regeneration niche. Climate factors that significantly affected germination included those that changed over the separation period between historic and recent seed collections. This indicates that short-time climatic changes may have the potential to impact germination in TDF woody species of southern Africa. The germination responses to climate factors were both positive and negative and it is not clear whether these are adaptations or not. Further research is required to assess the adaptive significance of these changes in germination traits in TDF species of southern Africa.

Introduction

Tropical dry forests (TDFs) are defined as forests and woodlands in tropical regions that experience alternating wet and dry seasons (Sankaran and Ratnam, 2013). The TDFs in east and southern Africa include the Zambezian Regional Centre of Endemism (ZRCE, White, 1983) and covers about 377 million ha in the southern Democratic Republic of Congo, Angola, Botswana, Malawi, Mozambique, Namibia, Tanzania, Zambia, Zimbabwe and the northeastern portion of South Africa. The climate in ZRCE is characterized by a 7–8 months long dry season, from April to November.

Climate change refers to a significant change in the trend of a climate variable, such as temperature or rainfall, over time, although it can also refer to a change in the frequency of climatic events, such as floods and droughts (measured as events per given period e.g., decade). Another aspect of climate change is variability, which refers to the degree of departure of a climate parameter from its mean value and this can occur even in the absence of a significant trend in a particular climatic variable.

Bonner (1984) defined some seed germination terms and Table 1 summarizes the relevant terms in relation to this study. Seed germination and seedling establishment are critical life stages in the sexual regeneration of plants. The regeneration process consists of multiple phases that range from flowering and pollination to fruit/seed formation, maturation, ripening and dispersal to seed germination and seedling establishment. The pre-dispersal phases are governed by the maternal environment (Sales et al., 2013; Fernández-Pascual et al., 2019; Kildishava et al., 2020). For example, the tissues surrounding the developing embryo, are mostly of maternal origin (Roach and Wulff, 1987). Some seed traits are also influenced by the parental genetic constitution (Copeland and McDonald, 1995). Climatic factors affecting the parent may therefore manifest themselves in the seed germination behaviour (Hudson et al., 2015). This implies that changes in the maternal genetic constitution and environment that are caused by climate change can alter germination traits and seedling recruitment.

Wyse and Dickie (2017) estimated that only 1.9% of the plant species in TDFs have desiccation-sensitive (recalcitrant) seeds which implies that many seed plants in TDFs of southern Africa have orthodox seeds that germinate easily when water and temperatures are favourable. However, viable seeds that fail to germinate when conditions for germination are favourable are usually considered to be dormant (Baskin and Baskin, 2004; Kildishava

Table 1 Description of terms used in this study based on Bonner (1984)

Term	Description
Seed	A matured ovule which contains an embryo and nutritive tissue and is enclosed in protective layers of tissue (seed coat)
Germination	A process that results in a seedling emerging above the soil surface
Germination fraction	Proportion of a seed collection that has germinated in a specified period, usually expressed as a percentage
Onset of germination	Week after last Saturday in November of each seed collection year when first emergent seedlings were observed
Duration of germination	The period (in weeks) between onset and end of germination
Peak germination	The week when germination was highest
Final germination	Cumulative germination at the end of the seasonal germination period

et al., 2020). According to Msanga (1998), out of 122 indigenous tree species in Tanzania, 70% are known to be non-dormant while 29% have physical dormancy (PY).

Climate has a strong influence on seed dormancy and germination (Walck et al., 2011). In general, the lower the temperature during seed formation, the higher the levels of PY dormancy during the seed development period (Roach and Wulff, 1987). However, the degree of PY dormancy can also vary with seed collection year and individual plants within a species (Smith et al., 2002). To assess differences in germination traits for dormant species, long term germination observations must be conducted at different time points after sowing (Soppe and Bentsink, 2020).

Dormancy allows seeds to travel in time and spread germination over years but climate change, especially warming, can shorten this travel time by enhancing loss of dormancy (Horvitz and Schemske, 1994; Ooi et al., 2009; 2014). For example, climate warming can alter natural cycles of seed dormancy (Ooi et al., 2009) and small increases in mean air temperature have been reported to cause increases in soil temperatures to much higher levels that can enhance loss of PY dormancy (Ooi et al., 2014). In fact, Everingham et al. (2021) reported that for every 5% increase in temperature variability there was a 3-fold increase in seed germination when comparing germination traits between historic and modern seed collections in Australia. It is important to note that unless the effect of a climate factor is specified with regards to the reproductive phenological phase of a species, it is difficult to identify the mechanism by which it affects regeneration from seed.

Resurrection approaches have been used to study effects of climate change on plants by comparing morphological and genetic traits of plants raised from long-term stored propagules and those raised from contemporary propagules of the same species originating from the same location (Franks et al., 2018). Such studies have involved long time intervals of many decades (Buswell et al., 2011; Guerin et al., 2012) but intervals of 10–30 years have also revealed that climate change can affect flowering phenology (Fitter and Fitter, 2002; Franks et al., 2007; Nevo et al., 2012) and other reproductive traits (Thomann et al., 2015; Sultan et al., 2021). However, despite climate change threats to biodiversity in southern Africa (Biggs et al., 2008; Chidumayo, 2008; Chidumayo and Sileshi, 2022) the author is not aware of any resurrection or time-separated studies into the effect of climate change on seeds of TDF species in southern Africa. The present study therefore assessed if germination traits in TDF woody species at a site in central Zambia, southern Africa, are responding to climate and climate change by comparing germination traits of seven species using time-separated historic seed collections (collected and sown during 1998–2005) and recent seed collections (collected and sown during 2016–2021). The study also aimed to assess whether such changes can be attributed to climate factors. The studied species included *Piliostigma thonningii* (Schumach.) Milne-Redhead and *Vachellia (Acacia) sieberiana* DC that are known to have physical dormancy (Sabiti and Wein, 1987; Ayisire et al., 2009; Mwase and Mvula, 2011). For these dormant species, an additional aim of the study was to assess whether climate factors affecting germination differed between seeds of the same collection and their cohort members that did not germinate in the first germination season but germinated after remaining in the soil for one or more years. The results have implications for the regeneration from seed of southern African TDF woody species under climate change.

Materials and methods

Study site

The study was conducted at a 5.0-ha site (15.47° S, 28.18° E, altitude 1260 m asl) in a disturbed TDF (Chidumayo, 2013) in the Makeni area of Chilanga district, about 15 km south of Lusaka city, Zambia, southern Africa. The climate in the area is subtropical with alternating dry (mid-March to mid-November) and wet (mid-November to mid-March) seasons and a mean annual precipitation of 796 mm. Mean daily monthly minimum and maximum temperatures are 15.7° and 24.8° C, respectively. The lowest and highest temperatures are experienced during May–August and September–November, respectively.

The soil at the study site is predominantly sand clay loam derived from limestone rocks and average texture composition in the top soil (0–30 cm depth) is 47% sand, 34% clay and 19% silt with a pH of 5.4 (Chidumayo, 2013). Soil nitrogen and organic matter content is 0.10% and 3.7%, respectively, while the concentration of available phosphorus is 2.7 mg kg^{-1} soil (Chidumayo, 2022). The site was fenced off in 1994 to keep out livestock and minimize undesirable human disturbances in the recovering disturbed forest.

Reproductive phenology and seed germination data

Phenology and seed germination were studied at the study site from 1998 to 2021 for seven woody species: *Erythrina abyssinica* Lam., (Papilionatae), *Lannea edulis* (Sond.) Engl., (Anacardiaceae), *Piliostigma thonningii* (Schumach.) Milne-Redhead, (Caesalpinioideae), *Securidaca longepedunculata* Fresen., (Polygalaceae), *Strychnos spinosa* Lam., (Loganiaceae), *Tamarindus indica* L. (Caesalpinioideae)

Table 2 Number of seeds sown per seed collection during the period 1998 to 2022 at the Makeni study site. Figures in brackets indicate separation period (years) between last historic and earliest recent seed collections per species

	Seeds sown per seed collection year									
	Historic period					Recent period				
Species	1998	2002	2003	2004	2005	2016	2018	2019	2020	2021
Erythrina (18)		200							70	80
Lannea (14)		450				350	200	100		
Piliostigma (12)	90	750	380	600		100		45	150	
Securidaca (16)				230					100	
Strychnos (15)	90	750	380	750	200				150	200
Tamarindus (15)			380		200				127	
Vachellia (15)			400	600				100	100	

and *Vachellia (Acacia) sieberiana* DC. (Mimosoideae). These species were selected because they had reproductively mature plants at the study site and in the surrounding area. From here on, the study species will be referred to by their genus names as *Erythrina, Lannea, Piliostigma, Securidaca, Strychnos, Tamarindus* and *Vachellia*. For each species, timing of phenological phases were recorded from mature plants throughout the study period with regards to leaf flushing, flowering, fruit development, ripening and shedding.

Ripe fruits and seeds of the study species were collected periodically, when available at the time of natural dispersal, at the study site and in the surrounding area. Seeds of all species, except *Lannea* and *Strychnos*, were removed from mature or dispersed fruits before storage until the onset of each wet season. The fleshy propagules of *Lannea* and *Strychnos* were first sun-dried to avoid fungal infections during storage (July-November). All seed collections were stored in aerated glass bottles under uncontrolled room conditions until planting time in November of each collection year. The number of seed collections and seeds sown per species depended on the availability of adequate current-year seeds (Table 2). Seeds were sown in sunny locations, away from tree canopies, in shallow holes (<5 cm deep) and covered lightly with soil in rows that were marked and mapped to facilitate subsequent inspections for seedling emergence which was used as a proxy for seed germination. Sown seeds were not subjected to any pre-treatment except for sun-drying in the case of *Lannea* and *Strychnos*. The number of seeds sown per planting station was recorded so that germination percentages could be calculated. Sowing was done on the last Saturday of November of each collection year. Seedling emergence was recorded every seven days from the time of sowing in November until the first week of April, after the end of the wet season in March. For species with PY dormancy, weekly recording of seedling emergence was also undertaken in subsequent wet seasons, following the initial sowing season, to obtain data on long-term seed germination over the subsequent years.

Local climate data

The local climate data used in this study were obtained from a meteorological station at Mount Makulu (15.55° S, 28.27° E, 1240 m asl), 13 km south of the study site. Mount Makulu (here after Mt Makulu) meteorological station was established in 1953 and weather data, including daily rainfall, minimum and maximum temperatures, have been recorded since then.

Data analysis

Germination data were divided into two periods: historic (1998–2005) and recent (2016–2021), separated by 12–18 years (mean = 15.0 years) (see Table 2). Germination fraction was calculated as emergent seedlings divided by sown seeds at weekly intervals starting from the last Saturday in November of each seed-collection year to determine the time of peak germination (see Table 1). Final germination (see Table 1) for each wet season (November – April) was derived by summing weekly germination fractions. For species with seed dormancy, germination in each subsequent wet season was based on weekly intervals starting from the last Saturday of November although seed sowing was done in the previous year(s). For easy monitoring and derivation of germination fractions for species with dormancy, the initial number of seeds sown (N) was maintained as the divisor in the calculation of germination fraction (GF) in the subsequent years as given in the following formula:

$$GF = R_{sy}/N$$

where R_{sy} is seedlings emerging in the subsequent year.

The significance of differences in germination fractions between historic and recent seed collections was determined using the Two-Proportions Fisher's test (Z-corrected) at $P = 0.05$.

Trends in weekly germination for each species in historic and recent seed collections were modelled and compared using the Loess regression analysis. If there were more than one data set for each species and period (see Table 2), the average germination was analysed. The Loess procedure fits smoothed curves and surfaces to multivariate scattered data using locally weighted regression. In a local regression model, the dependent and independent variables are related as follows:

$$y_i = g(x_i) + e_i$$

where g is the regression surface and e_i are random errors. The underlying theory is that in a given neighbourhood of a point in the *x*-space, the surface can be approximated using either linear or quadratic polynomials. For any x_i, the fitted value for y_i

is computed using only those cases where the values of the independent variable are in the neighbourhood of x_i and those closest to x_i are given greater weight. The Alpha value of the neighbourhood parameters was set at 0.75 because this gave the best residual plots for the model. The regression was fitted using quadratic polynomials because these gave the lowest residual standard error and highest coefficient of determination (R^2).

Simple relationships between final germination percentage for each specific seed collection and climate factors (independent variables) were assessed using linear regression. The climate factors assessed were (i) monthly rainfall, (ii) monthly average daily maximum temperature and (iii) monthly average daily minimum temperature. Best subset linear regression analyses were used to select the most important climate factor that explained the largest variance in germination in each species. When two independent variables are highly correlated the analytical procedure used automatically drops one of the independent variables to avoid problems of collinearity and over-fitting. Best subset regression analysis simultaneously compares models with single variables and all their possible combinations. The model with the lowest Akaike's Information Criterion for small samples (AICc) and highest coefficient of determination (R^2) was selected as the best model (Burnham and Anderson 2002). This analysis was also applied to germination of dormant seeds that occurred during subsequent years after initial sowing.

Trends in climate factors over time at Mt Makulu weather station were explored using linear regression analysis for the period 1953 to 2021 and simple mathematical comparisons between historic (1998–2005) and recent (2016–2021) times. All statistical analyses were done in Statistix 9 (Analytical Software 1985–2008).

Results

Reproductive phenology

Ripe fruits were shed in the dry season from July to October, except in *Erythrina* and *Lannea* in which propagule dispersal occurred in November and early December (Fig. 1). These two species also had the shortest reproductive phenology and fruits developed and matured in less than two months and flowering preceded leaf flush (Fig. 1). In the other five species, fruit development and maturation took eight to ten months and flowering either coincided with leaf flush or occurred soon after leaf flush (Fig. 1).

Germination of the non-dormant study species

Piliostigma and *Vachellia* are the only species that continued to germinate in subsequent wet seasons after sowing and are therefore considered as dormant species and are treated separately (see next subsection). The remaining five species only germinated in the season of sowing and are therefore considered as non-dormant species. The pattern in weekly germination between historic and recent seed collections varied among the five species: in all the five species peak germination was lower in recent seed collections compared to historic seed collections (Fig. 2). In *Securidaca* the time of peak germination occurred earlier while in *Strychnos* this occurred later among recent seed collections (Fig. 2).

Final germination decreased in all the five non-dormant species from historic to recent times while the onset of germination did not change in four species but this occurred earlier in recent seed collections in *Lannea* (Table 3). Duration of germination was longer for recent seed collections in *Erythrina*, *Lannea* and *Strychnos* but was shorter in *Securidaca* and *Tamarindus*.

Rainfall in March, April and October explained a significant proportion of the final germination variance in *Securidaca*, *Tamarindus* and *Lannea*, respectively (Table 4). In both *Lannea* and *Securidaca* more rainfall increased germinability. However, more rainfall in April reduced the germinability of *Tamarindus* seed which occurred in the middle of the fruit development period (see Fig. 1). In *Strychnos*, maximum temperature in October had a significant negative effect on seed germinability and this occurred at the time of leaf flush and flowering (see Fig. 1). Minimum temperature in June had a significant negative effect on seed germinability in *Erythrina* and the timing coincided with the onset of leaf shedding in *Erythrina* (not shown in Fig. 1).

Germination in species with seed dormancy

In *Piliostigma* overall germination increased from 4.8% for seeds without prior burial to 16.6% after one year in the soil, before
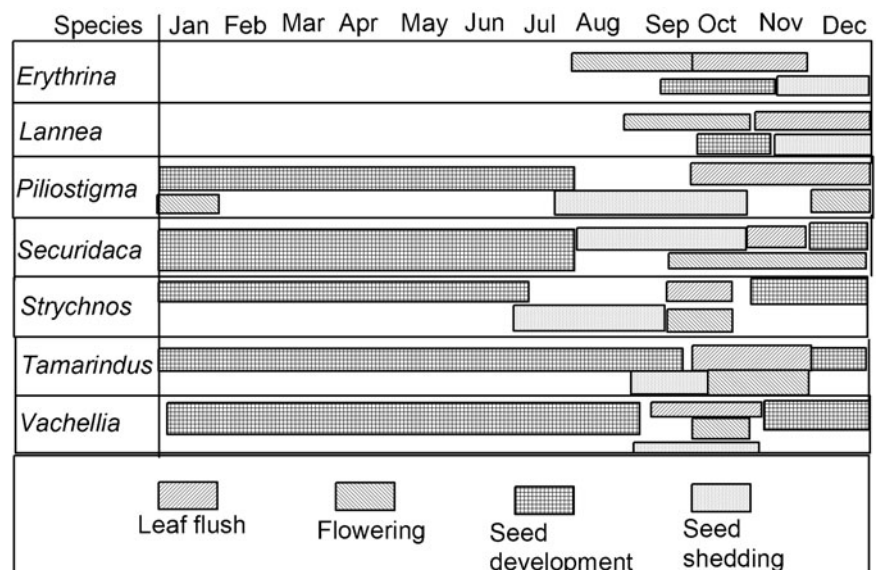

Figure 1. Species phenological calendar at the study site and the surrounding area.
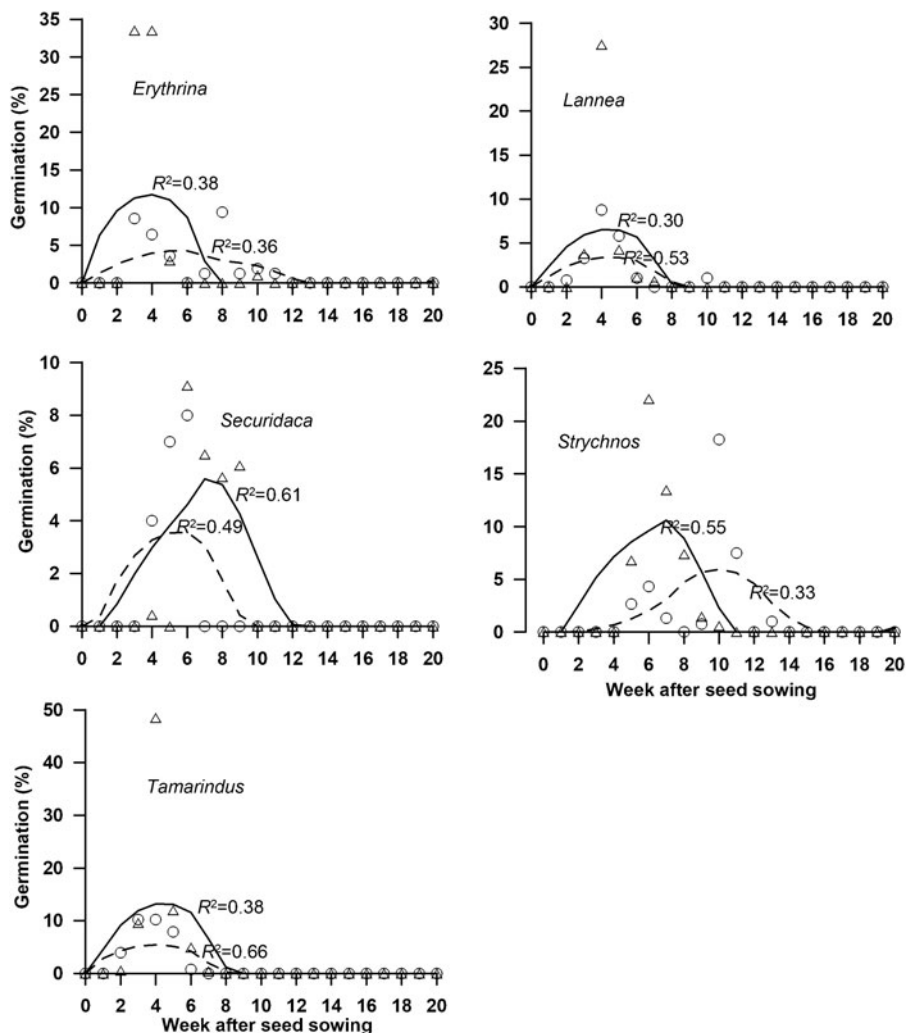
Figure 2. Weekly germination patterns in historic (triangles) and recent (circles) seed collections in five non-dormant species. Fitted Loess models for historic (solid lines) and recent (dash lines) seed collections.

decreasing to 2.7%, 0.6% and 0.4% after two, three and four years, respectively. For historic seed collections, the final germination percentage of 5.1% without burial was significantly lower than that of 15.2% for seeds buried for one year ($Z = -10.89$, $P < 0.0001$). Similarly, for recent seed collections, the final germination percentage of 0.7% for seeds without burial was significantly lower than that of 36.0% for seeds buried for one year ($Z = -8.00$, $P < 0.0001$). In *Vachellia* overall final germination decreased from

Table 3 Changes in germination traits between historic and recent seed collections of non-dormant species at the study site. The values were obtained by subtracting historic from recent time period

| Species | Change in germination traits between historic and recent seed collections | | | |
	Onset (weeks)	Peak (%)	Final (%)	Duration (weeks)
Erythrina	0	−24.13	−37.43	1
Lannea	−1	−18.82	−16.76	4
Securidaca	0	−1.13	−8.83	−3
Strychnos	0	−3.88	−16.00	3
Tamarindus	0	−38.29	−42.94	−1

25.5% without prior burial to 1.8% and 0.1% after one and two years in the soil, respectively. Among historic seed collections, the final germination percentage of 26.4% prior to burial was significantly higher than that of 2.0% among recent seed collections ($Z = 8.98$, $P < 0.0001$). However, the reverse was true for seeds buried for one year in which the final germination of 0.5% among historic seed collections was significantly lower than that of 38.0% for recent seed collections ($Z = -3.21$, $P = 0.001$).

Peak weekly and final germination decreased marginally by <1.0% and 3.2%, respectively, in *Piliostigma* from historic to recent times. In *Vachellia* peak weekly germination decreased by nearly 12% from the historic levels while the decrease in final germination was 43%. The onset of germination did not change in *Vachellia* but this occurred later, by four weeks, in *Piliostigma* for recent seed collections. Duration of germination was shortened by nine weeks in both *Piliostigma* and *Vachellia* among recent seed collections.

The bimodal weekly germination patterns for seeds without prior burial were maintained for both historic and recent seed collections in *Piliostigma* but after one year burial, the germination pattern was unimodal and peak germination occurred later in recent seed collections (Fig. 3). In *Vachellia* seeds without prior burial, the peak germination had virtually disappeared among recent seed collections compared to historic seed collections (Fig. 3). However, after one year in the soil, the germination

Table 4 Final germination among seed collections and determinant climate factors for the five non-dormant species. Climate determinant factors are abbreviated as follows: first three letters refer to month, MaxT is maximum temperature (°C), MinT is minimum temperature (°C) and Rfll is rainfall (mm)

Species	Mean germination (±1 SE, %)	Determinant climate factor	Variance explained (R^2, %)	F-value	Significance level (P)	Impact
Erythrina	48.7 ± 8.4	JunMinT	93	26.04	0.04	Negative
Lannea	20.7 ± 4.7	OctRfll	32	6.44	0.02	Positive
Securidaca	22.8 ± 2.6	MarRfll	89	24.29	0.02	Positive
Strychnos	44.5 ± 9.9	OctMaxT	88	22.28	0.02	Positive
Tamarindus	63.4 ± 15.7	AprRfll	94	31.74	0.03	Negative

pattern in *Vachellia* reversed with recent seed collections having a higher peak than those in the historic seed collections (Fig. 3).

In *Piliostigma* seeds without prior burial, minimum temperature in September significantly and negatively affected final germination but after one year in the soil, minimum temperature in August explained 71% of germination variance (Table 5). After two years in the soil, it was maximum temperature in January that explained 93% of germination variance with positive effects on germination. In *Vachellia* seeds without prior burial, final germination was significantly and negatively affected by maximum temperature in February and minimum temperature in June but after one year in the soil, only minimum temperature in June explained 94% of the germination variance (Table 5). This effect was positive with an increase in temperature resulting in an increase in final germination.

Local climate trends and significant factors

Annual and monthly rainfall at Mt Makulu showed no significant trends from 1953 to 2021 but annual rainfall coefficient of variation (CV) increased from 17.5% during 1953 to 1989 to 25.4% during 1990 to 2021. Annual maximum temperature increased significantly from 1953 to 2021 ($R^2 = 0.46$, $F = 44.23$, $P < 0.0001$). Similarly, significant increases have occurred in maximum temperatures in November ($R^2 = 0.22$, $F = 6.22$, $P = 0.02$) and January ($R^2 = 0.25$, $F = 7.66$, $P = 0.01$) but the trends for the other months were not significant. Linear regression analysis revealed a significant increase in annual minimum temperature ($R^2 = 0.08$, $F = 4.63$, $P = 0.04$) although a 3-degree polynomial model gave a higher significance ($R^2 = 0.24$, $F = 5.20$, $P = 0.003$). The latter indicated a non-linear trend consisting of an increasing trend from mid-1960s to mid-2000s and a decreasing trend thereafter. However, minimum temperature showed a significant decreasing trend for February ($R^2 = 0.25$, $F = 7.83$, $P = 0.01$), March ($R^2 = 0.40$, $F = 15.51$, $P = 0.001$) and April ($R^2 = 0.23$, $F = 6.67$, $P = 0.02$) but no significant trend was observed for the other months. Depending on the species, seed collections were stored from July to November each year and room climatic conditions during storage are assumed to have been similar between historic and recent times although open-air maximum temperature in November has increased from 1953 to 2021 (see above).
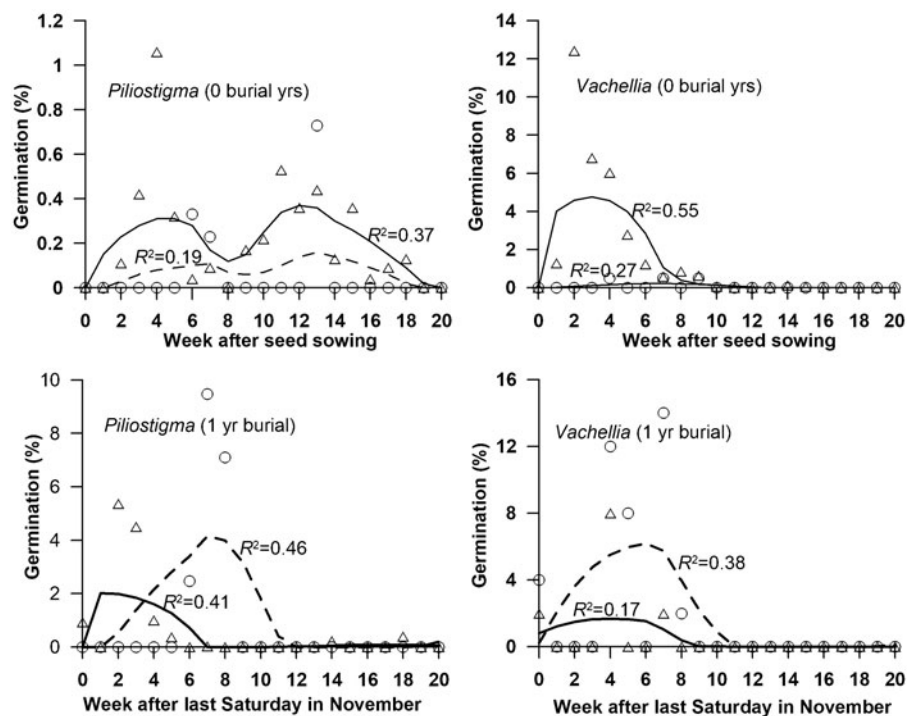

Figure 3. Weekly germination patterns in historic (triangles) and recent (circles) seed collections in *Piliostigma* and *Vachellia* without prior burial (top panel) and after one year in the soil (bottom panel). Fitted Loess models for historic (solid lines) and recent (dash lines) seed collections.

Table 5. Final germination among seed collections and determinant climate factors for the two dormant species. Climate determinant factors are abbreviated as follows: first three letters refer to month, MaxT is maximum temperature ($^{\circ}$C), MinT is minimum temperature ($^{\circ}$C).

Species	Treatment	Determinant climate factor	Variance explained (R^2)	F-value	Significance level (P)	Impact
Piliostigma	No prior burial	SepMinT	35%	4.94	0.05	Negative
	One year burial	AugMinT	71%	10.00	0.03	Negative
	Two years burial	JanMaxT	93%	24.68	0.04	Positive
Vachellia	No prior burial	JunMinT & FebMaxT	86%	12.13	0.02	Negative
	One year burial	JunMinT	94%	61.06	0.001	Positive

Of the three monthly-climate factors that had a significant effect on seed germination of the study species, only monthly daily maximum temperature in January had a long-term significant increasing trend at Mt Makulu (see above). Rainfall in October and March has decreased in recent times but variability (CV) for October increased by nearly 86% compared to –5.73% in March (Table 6). Rainfall in April has undergone the least change between historic and recent times (Table 6). Variability in monthly daily minimum temperatures in June, August and September increased in recent times (Table 6). Monthly daily maximum temperature in January and February increased slightly in recent times but the increase of 1.7° CV was higher for maximum temperature in October (Table 6). Although maximum temperature in October increased in recent times, monthly rainfall decreased while its variability increased.

Discussion

Germination in non-dormant species

The historic and recent periods on which this study is based are separated by 12–18 years. Rapid evolution over an 11-year period has been reported for *Polygonum cespitosum* (Sultan et al., 2021) and germination of *Centaurea cyanus* increased by 18% over an 18-year period (Thomann et al., 2015). In this study, peak germination decreased and its timing occurred earlier in recent seed collections of *Securidaca* but occurred later in *Strychnos*. Final germination also decreased in all the non-dormant species. The combination of lower peak and final germination percentages

implies that seedling recruitment decreased with negative consequences on regeneration from seed for these five woody species. The onset of germination did not change during the separation period, except in *Lannea* where this had advanced in recent times. Generally, germination duration in recent times was longer in *Erythrina*, *Lannea* and *Strychnos* while this was shorter in *Securidaca* and *Tamarindus*. However, changes to the duration of the germination period did not alter the negative effects of lower peak and final germination on seedling demography in these species.

The determinants of final germination were rainfall in October in *Lannea*, March in *Securidaca* and April in *Tamarindus*. The timing of these determinants was in the middle of the seed development period and therefore may have impacted the maternal environment during a critical reproductive phase. Indeed, conditions experienced by developing seeds in the mother plants have been reported to affect seed germination (Sales et al., 2013). Rainfall in October has decreased in recent times, but more importantly, its variability has almost doubled, thereby creating stressful conditions during seed development which may have contributed to the lower germination in *Lannea*. Rainfall in March has also decreased by nearly 41% in recent times and higher rainfall enhanced seed germination in *Securidaca*. The decreasing rainfall in March in recent times may therefore negatively affect germination in *Securidaca*. Higher rainfall in April had a negative effect on germination in *Tamarindus* and rainfall in April in recent times has increased by 43% which is likely to reduce seed germination in this species.

In *Strychnos*, maximum temperatures in October had a significant negative effect on seed germinability and this occurred

Table 6 Changes in determinant monthly climate factors with significant effects on final seed germination in the seven study species from historic to recent times. Climate factors are abbreviated as follows: first three letters refer to month, MaxT is maximum temperature ($^{\circ}$C), MinT is minimum temperature ($^{\circ}$C) and Rfll is rainfall (mm)

Climate factor	Average (±1SE) for each period			Coefficient of variation (CV) for each period		
	Historic	Recent	Change	Historic	Recent	Change
OctRfll	24.47 ± 7.0	6.48 ± 4.28	−17.99	75.69	161.8	86.11
MarRfll	135.5 ± 41.48	70.99 ± 18.59	−64.51	74.98	69.25	−5.73
AprRfll	21.43 ± 12.07	30.41 ± 14.81	8.98	137.97	128.84	−9.13
JunMinT	7.94 ± 0.23	8.76 ± 0.44	0.82	7.63	13.26	5.63
AugMinT	11.19 ± 0.42	12.83 ± 0.95	1.64	9.86	18.14	8.29
SepMinT	15.54 ± 0.47	15.80 ± 0.60	0.26	7.94	9.32	1.38
JanMaxT	28.37 ± 0.39	29.14 ± 0.56	0.78	3.61	5.09	1.48
FebMaxT	28.07 ± 0.22	28.39 ± 0.32	0.32	2.06	2.97	0.91
OctMaxT	32.49 ± 0.79	34.17 ± 0.41	1.68	6.43	2.96	−3.47

during flowering/pollination time. The high maximum temperatures in October possibly contributed to the low pollination and/or fertilization which can affect seed germination. It is not clear whether this is linked to inefficient pollination by pollinators or not. Further research is required to clarify this possible linkage. Maximum temperatures in October have increased by 1.68° C in recent times and this may have contributed to the reduced seed germination in *Strychnos*. Minimum temperature in June had a significant negative effect on seed germinability in *Erythrina*. June coincides with the onset of leaf shedding in *Erythrina*. The decrease in nutrient content in senescing leaves of TDF trees has been attributed to nutrient reabsorption which occurs before leaf shedding (Ernst, 1975; Tolsma et al., 1987; Chidumayo, 1994). Fruiting frequency in *Isoberlinia angolensis* and *Julbernardia globiflora* in TDFs of Zambia was linked to the allocation of nitrogen and potassium to reproduction (Chidumayo 1997). It is probable that minimum temperatures in June reduce nutrient reabsorption efficiency that subsequently results in low nutrient remobilization to support seed development which occurred before leaf flush in *Erythrina* and is therefore dependent on stored resources. However, minimum temperatures in June have increased by 0.82° C in recent times which may improve seed germination in this species.

Germination in species with seed dormancy

Final germination in *Piliostigma* was highest after one year in the soil and tailed off sharply in the subsequent three years. This germination pattern was similar for both historic and recent seed collections. Thus, soil conditions in the first year after sowing may have greatly increased dormancy loss in *Piliostigma*. Germination of *Piliostigma* during the first wet season was bimodal which separates seeds that germinate early (December – January) from those that germinate late (February – April) in the season. It is apparent that there are two seed morphs among first wet season germinators: early and late germinators. This germination pattern represents bet-hedging although its adaptive value is not clear because rainfall pattern at the site is unimodal and seedling survival is not related to time of germination (Chidumayo, 2008). There was a major shift in the germination pattern of *Piliostigma* from a bimodal among seeds without prior burial to a unimodal in seeds buried for one year, and for the latter, peak germination occurred earlier in historic times than in recent times. This shift may be due to the dilution of maternal effects on germination after one year burial and the increasing role of direct environmental factors in influencing germination among buried seeds. This proposition is supported by the shifts in significant factors affecting final germination in *Piliostigma* from minimum temperature in September in seeds without prior burial to minimum temperature in August after one year in the soil and maximum temperature in January after two years in the soil. Along with these changes, there was also an increase in the variance explained that increased progressively along this time line from 35% to 71% and 93%. September is towards the end of seed maturation and minimum temperature during this period may indicate the role of maternal temperatures in influencing germination in *Piliostigma*. This observation also supports the idea that lower temperatures during seed development affect levels of dormancy during seed maturation (Roach and Wulff, 1987). In fact, high minimum temperatures in September were observed to reduce final seed germination in *Piliostigma* perhaps due to a higher degree of dormancy.

After one year in the soil, high minimum temperatures in August reduced germination which suggests that low minimum temperatures in August are required to prime buried seeds for enhanced germination in *Piliostigma*. However, high maximum temperatures in January may be prerequisites for high germination in seeds that have been buried for two years. Ernst et al. (1988) reported that high temperatures under natural conditions improved germination in TDF tree species. It is important to note that peak germination in buried *Piliostigma* seeds occurred in January which indicates an increasing dominance of early-season germinators over time in recent times.

In *Vachellia* historic seed collections without prior burial, had the highest final germination but in recent seed collections the highest final germination occurred after one year in the soil. There appears to have been a dramatic shift in seed dormancy in *Vachellia* from historic times when dormancy was low to a higher degree of dormancy in recent times that required one year of burial to break.

Results suggest that maximum temperature in February and minimum temperature in June have a joint negative effect on the germination of *Vachellia* prior to burial. Both factors operated during seed development in the mother plants and such factors have been reported to affect germination (Sales et al., 2013). This implies that low maximum temperatures in February and minimum temperatures in June may be required to produce seed with a high germinability in *Vachellia*. One of these factors also appears to affect seed dormancy. Maximum temperature in February has increased by 0.32 °C while minimum temperature in June has increased by 0.82 °C in recent times (see Table 6). The degree of seed dormancy in *Vachellia* has also increased in recent seed collections with little or no germination occurring in seeds without prior burial (see Fig. 3). In a Neotropical tree, *Dimorphandra mollis*, germination only occurred after at least one year of burial (Borghetti et al., 2019). However, after burial for one-year, minimum temperature in June is the only factor that significantly affected germination in *Vachellia* and higher minimum temperatures in June appeared to improve germinability. Consequently, minimum temperature in June affected *Vachellia* seeds differently in the mother plants and when buried in soil.

Climate change and germination in African TDF woody species

Of the five monthly climate variables that had significant trends at Mt Makulu over the 1953–2021 period, only maximum temperature in January significantly affected germination in *Piliostigma* seeds that had been buried for two years. Seed germination in the study species appeared to respond to climate changes at shorter time periods, such as those observed between 1998–2005 and 2016–2021 (see Table 6). Studies involving short time periods have revealed significant changes in plant reproductive traits to short-term changes in climate factors (Fitter and Fitter, 2002; Franks et al., 2007; Nevo et al., 2012; Thomann et al., 2015; Sultan et al., 2021). In the case of rainfall in October, both a decrease in rainfall and increase in its variability appeared to significantly affect germination in *Lannea*. In fact, Everingham et al. (2021) reported that for every 5% increase in temperature variability there was a 3-fold increase in seed germination when comparing germination traits between historic and modern seed collections in Australia. Clearly, long term trends in climate factors are useful in studying responses by plants to climate change but such responses can also be observed over shorter time periods

even in the absence of long-term climate trends, as shown by this study.

In non-dormant species and during the first germination season among dormant species, germination was largely driven by the environment experienced by mother plants. Seed germination is a property acquired primarily from maternal resources allocated to the seed and utilized by the seed in the germination niche (Roach and Wulff, 1987). These resources are passed to the offspring during the pre-dispersal reproductive stages. Physical dormancy is a trait acquired by the seed during ripening from the mother plants. Both *Piliostigma* and *Vachellia* that have physical dormancy (Sabiti and Wein, 1987; Ayisire et al., 2009; Mwase and Mvula, 2011) germination after the first germination season is also controlled by factors operating at the germination niche that perhaps assume more importance over maternal factors. Factors such as maximum and minimum temperatures appeared to drive germination in dormant seeds that germinated after one- and two-years following sowing. Warming is reported to enhance loss of physical dormancy (Horvitz and Schemske, 1994; Ooi et al., 2009; 2014) and small increases in mean air temperature have also been observed to cause increases in soil temperatures to much higher levels that can enhance loss of PY dormancy (Ooi et al., 2014).

However, it is important to note that plant responses to climate factors are subject to year-to-year variations that can only be captured in multi-year seed collections during each specific time period. In this study, *Erythrina*, *Lannea* and *Securidaca* were represented by single seed collections for the historic period and *Securidaca* and *Tamarindus* were also represented by single seed collections for the recent period (see Table 1). Therefore, the results for these species and periods do not incorporate inherent year-to-year variation in germination and should be treated with caution until further studies with more seed collections have been conducted to confirm the present findings.

Conclusion

This study used time-separated seed collections and sown in the wet season following collection during 1998–2005 (historic period) and 2016–2021 (recent period) to assess whether germination traits in seven TDF woody species had changed during the separation period and whether the changes were in response to climate factors. In all the species, peak and final germination were lower in recent (2016–2021) seed collections compared to historic (1998–2005) seed collections during the first germination season. However, in species with PY dormancy, both peak and final germination after one year burial were higher in recent seed collections than historic seed collections. The findings indicate that climate factors significantly contributed to the observed changes in germination traits in the study species. Germination in the first germination season appeared to be controlled by the maternal environment during the pre-dispersal reproductive stages, especially during the seed ripening period. In species with seed dormancy, post-first-year germination responded to climate factors in the seed germination niche. Climate factors that significantly affected germination, were not necessarily those with long-term change trends but included those that changed over the short separation period of 12–18 years. This indicates that short-time climatic changes also may have the potential to impact germination in TDF woody species in southern Africa. The germination responses were both positive and negative and it not clear whether these responses are adaptive or not.

Further research is required to determine the adaptive value of these responses in TDF woody species in southern Africa.

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