

Die-off of giant *Euphorbia* trees in South Africa: Symptoms and relationships to climate

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Received 24 February 2012; received in revised form 2 August 2012; accepted 24 August 2012

Abstract

Euphorbia ingens is the largest of the succulent tree Euphorbias in Southern Africa. In South Africa, it is most abundant in the northern portion of the country, especially in the Limpopo and North West Provinces. In the mid-nineties, *E. ingens* were noted to show symptoms of disease, and by 2000 severe mortality had begun to occur in the Limpopo Province. Various factors have been suggested as possible causes of this mortality. We investigated the possible involvement of changes in climate in the sudden die-offs of *E. ingens*. Four sites within the severely affected Limpopo Province and two sites in the less affected North West Province were included. Nine linear transects were established at each site. Each tree within a transect was scored as alive or dead, and as mature or juvenile. Insect and disease symptoms, and environmental variables were also evaluated. Trees in the Limpopo Province were more severely affected by disease and insects and exhibited higher levels of mortality compared to trees in the North West Province. Analyses of weather data revealed greater upward trends in temperature and downward trends in precipitation in the Limpopo Province compared with the North West Province. Estimates of potential evapotranspiration and water balance indicated an increase in water demand while precipitation has remained the same or has decreased. The dramatic degree of mortality of *E. ingens* that has been observed since about 2000 appears to be linked to increasing moisture deficits resulting in tree stress, which in turn allows opportunistic fungal pathogens and insects to increase in severity, ultimately contributing to tree mortality.

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Keywords: Climate change; *E. ingens*; Mass mortality; Potential evapotranspiration; Water balance

1. Introduction

Euphorbia ingens E. Meyer: Boissier is a large, woody, succulent tree known only from Africa where it has a wide distribution across Botswana, Lesotho, Malawi, Mozambique, South Africa, Swaziland and Zimbabwe. This species occurs in high densities in localized sites in the northern provinces of South Africa (Gauteng, KwaZulu-Natal, North West and Limpopo) with especially high abundances in the Limpopo

Province (Gildenhuys, 2006; Palgrave et al., 2002; Van Wyk and Van Wyk, 1997). In the last 10 to 15 years, high levels of mortality of *E. ingens* have been observed in the Limpopo Province (Malan, 2006; Roux et al., 2008, 2009). No previous declines or die-offs of *E. ingens* are known to have occurred. Therefore, this striking and rapid mortality is of great concern. While the cause of the mortality is not known, insects, pathogens and climate change have all been speculated to play a role (Malan, 2006; Roux et al., 2008, 2009).

Globally, tree die-offs have been increasingly reported since the 1970s (Allen et al., 2010; Raffa et al., 2008) and are affecting various tree species (Fensham and Holman, 1999; Fisher, 1997; Foden et al., 2007; Hogg et al., 2008; Suarez et al., 2004; Tsopelas et al., 2004). Die-offs have occurred in a variety of ecosystems

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indicating that this phenomenon is not limited to one type of environment (Allen et al., 2010). Tree mortality is increasingly being linked to the occurrence of drier conditions, or more variable rainfall patterns, due to climate change (Allen, 2009; Bigler et al., 2006; Clark, 2004; Dezzio et al., 1997; Jurskis, 2005; Liang et al., 2003; Lwanga, 2003; Wardle and Allen, 1983). Drier conditions can lead directly to mortality through effects on tree physiological functions, or indirectly through effects on tree vigor and defenses, which allow insects and pathogens to kill trees more easily and even develop widespread outbreaks (Allen et al., 2010; Konkin and Hopkins, 2009).

Shifting precipitation patterns are accompanying warming in many regions globally (Bergstrom and Chown, 1999; King and Harangozo, 1998; Peters, 1990; Pounds et al., 1999). Such shifts can result in precipitation occurring at times other than when trees most need it, creating physiological stress, and lowered resistance to insects and disease, which may ultimately lead to increased levels of tree mortality (Dukes et al., 2009; La Porta et al., 2008; Sinclair and Lyon, 2005; Tubby and Webber, 2010). However, in areas experiencing no change in amount or timing of precipitation, warming may still result in stress, and ultimately mortality, due to longer growing seasons and higher demand for water with no concurrent increase in moisture (Dale et al., 2001; Sinclair and Lyon, 2005; Stone et al., 2007; Sturrock et al., 2011).

Initial studies on the die-off of *E. ingens* were conducted at the Biodiversity Conservation Centre of the National

Zoological Gardens (NZG), Mokopane, Limpopo Province, South Africa, where the main symptom of disease in affected trees was a gray discoloration of normally green succulent branches (Malan, 2006). In that study, Malan (2006) was unable to link symptom development and eventual tree death to alien plant invasion, precipitation (due to limited data), fire, or soil erosion. The possibility that insects or pathogens were responsible for the die-off led to a more detailed study at the same site to investigate the potential role of these factors (Roux et al., 2008, 2009). Various symptoms of disease were found on trees at the site, including blue stain of the main woody stems of diseased trees, as well as spots and subsequent rotting of the succulent branches. Insect infestations were also found in both the woody and succulent parts of the plants (Roux et al., 2008, 2009). From affected portions of trees, fungi from the Botryosphaeriaceae, Microascales, Ophiostomataceae and Teratosphaeriaceae were isolated and insects in the Pyralidae and Curculionidae were collected (Roux et al., 2008, 2009; Van der Linde et al., 2012a, b). However, while a number of fungi and insects were found infesting affected trees, none appeared to be the primary cause of mortality.

Because no single insect or pathogen stood out as being a major driver in *E. ingens* mortality, we hypothesized that climate change (changes in temperature and/or precipitation regimes) may be a primary cause of decline and mortality of *E. ingens* in Southern Africa. Anthropogenic warming is already occurring in Africa where temperatures are predicted to increase

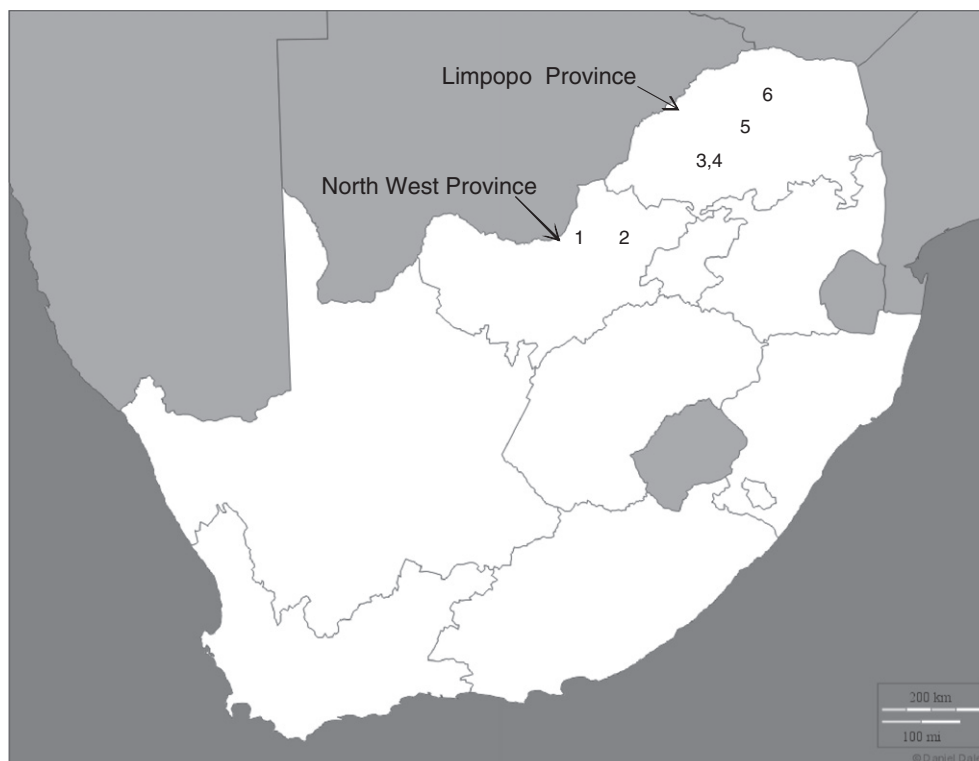


Fig. 1. Map of South Africa showing locations of study sites used to investigate die-off of *Euphorbia ingens*. 1 = Enzelsberg, 2 = Wolfaan, 3 = Euphorbia drive, 4 = Homestead, 5 = Capricorn and 6 = Last Post.

Table 1
Characteristics and locations of study sites investigating die-off of *Euphorbia ingens* in the Limpopo and North West Provinces, South Africa.

Site name	GPS coordinates	Elevation (m)	Locality	Density of savanna vegetation	Soil	Rainfall station (distance from site in km)	Temperature station (distance from site in km)
Enzelsberg (1)	S25 22.817 E26 15.975	1100–1200	Enzelsberg	Very dense	Rocky/clay	Tuscany (16)	Marico (17)
Wolfaan (2)	S25 46.411 E27 36.404	1200–1300	Rustenburg	Dense	Sandy	Brits Hartbeespoortdam (19)	Buffelspoort II AGR (18)
Euphorbia drive (3)	S24 09.923 E29 02.275	1200–1300	Mokopane	Sparse	Clay	Palmer Estate (6)	Mokopane (17.5)
Homestead (4)	S24 10.437 E29 03.266	1200–1300	Mokopane	Dense	Clay	Palmer Estate (6)	Mokopane (17.5)
Capricorn (5)	S23 21.910 E29 44.621	1000–1100	Louis Trichardt	Sparse	Sandy	Mara-Pol (29.5)	Mara (49)
Last Post (6)	S23 17.738 E29 55.467	900–1000	Louis Trichardt	Sparse to dense	Rocky/clay	Mara (39.5)	Mara (39.5)

by three to seven degrees Celsius by the year 2100. Concurrently, rainfall is expected to either increase or decrease, depending on region, by approximately 20% during the 21st century (Boko et al., 2007; Engelbrecht, 2011; Houniet et al., 2009). Changes in environmental conditions, such as changing temperatures and rainfall amounts or timing may stress *E. ingens* trees, particularly those occurring at their ecological margins, leading directly to mortality through physiological stress or indirectly through responses by secondary insects and pathogens.

The objectives of this study were to determine whether temperature or precipitation patterns are related to insect activity, symptoms of disease, and degree of mortality of *E. ingens* in

South Africa. Our study was an attempt to determine the main factors underlying the die-off of *E. ingens*.

2. Materials and methods

2.1. Study sites

The study was conducted in South Africa from August 2009 to April 2010 at sites in the Limpopo Province (Euphorbia drive, Homestead, Last Post and Capricorn), where the die-off is very severe, and the North West Province (Enzelsberg and Wolfaan), where the die-off of *E. ingens* trees is minimal

Table 2
Mortality, % mature vs juvenile trees, and scores for symptoms on live trees in each transect at each study site.

Site	Mortality (%)	Mature (%) ^a	Gray (mean rank) ^b	No. of trees with gray discoloration (%)	Moth damage ^c (mean no. of trees per transect)	No. of trees with moth damage (%)	Spots ^d (mean no. of trees per transect)	No. of trees with spots (%)
Enzelsberg total no. trees in transects=224	13	58	0.03	4	14	55	17	67
Wolfaan Total no. trees in transects=627	5	46	0.20	16	5	7	46	67
Euphorbia drive Total no. trees in transects=189	22	99	1.90	72	16	78	16	78
Homestead Total no. trees in transects=277	36	83	1.10	64	13	43	18	60
Capricorn Total no. trees in transects=182	1	60	0.55	40	9	42	15	74
Last Post Total no. trees in transects=172	2	94	1.3	93	12	61	18	95

No scores were obtained for transect eight, since all the trees in this site were dead.

^a Mature: refers to percentage of adults to juveniles within each transect.

^b Gray: gray discoloration occurring on succulent branches rated from 0 (lowest)–3 (highest).

^c Moth damage: internal feeding on succulent branches (rated as present/absent).

^d Spots: white spotting occurring on the succulent branches (rated as present/absent).

Table 3

ANOVA tables for comparisons of monthly mean maximum and minimum temperatures (°C), and precipitation (mm) for four decades (1969–1978, 1979–1988, 1989–1998, 1999–2008) at each of five sites in South Africa used in this study. For least square means for a particular ANOVA, values followed by a different letter are significantly different at $P < 0.05$.

Source of variation	df	F	P	Least square means (SE) by decade	
<i>Enzelsberg</i>					
Maximum temperature					
Decade	3	8.29	<0.001	69–78	26.89 (0.18)a
Decade × month	33	0.85	0.71	79–88	27.93 (0.17)b
Total	359			89–98	27.52 (0.17)b
				98–08	26.86 (0.18)a
Minimum temperature					
Decade	3	3.59	0.01	69–78	11.72 (0.13)a
Decade × month	33	0.47	0.99	79–88	12.29 (0.14)b
Total	347			89–98	11.77 (0.12)a
				99–08	11.81 (0.12)a
Precipitation					
Decade	3	3.14	0.03	69–78	52.80 (4.12)a
Decade × month	33	1.33	0.11	79–88	42.97 (4.12)a
Total	479			89–98	55.26 (4.12)a
				99–08	46.58 (4.12)a
<i>Wolfaan</i>					
Maximum temperature					
Decade	3	5.07	0.002	69–78	26.09 (0.14)a
Decade × month	33	1.02	0.44	79–88	26.10 (0.14)a
Total	479			89–98	26.14 (0.14)a
				99–08	26.75 (0.14)b
Minimum temperature					
Decade	3	60.72	<0.001	69–78	11.08 (0.16)a
Decade × month	33	0.88	0.67	79–88	11.20 (0.16)a
Total	478			89–98	11.50 (0.16)a
				99–08	13.68 (0.17)b
Precipitation					
Decade	3	0.51	0.68	69–78	54.87 (4.15)a
Decade × month	33	0.89	0.65	79–88	50.93 (4.15)a
Total	477			89–98	57.18 (4.17)a
				99–08	57.28 (4.17)a
<i>Euphorbia dr./Homestead</i>					
Precipitation					
Decade	3	0.54	0.65	69–78	48.27 (3.36)a
Decade × month	33	1.54	0.03	79–88	46.32 (3.35)a
Total	476			89–98	42.33 (3.37)a
				99–08	46.06 (3.35)a
<i>Capricorn</i>					
Maximum temperature					
Decade	3	11.75	<0.001	69–78	26.74 (0.13)a
Decade × month	33	0.82	0.76	79–88	27.36 (0.13)b
Total	479			89–98	27.45 (0.13)bc
				99–08	27.85 (0.13)c
Minimum temperature					
Decade	3	8.40	<0.001	69–78	12.08 (0.10)a
Decade × month	33	0.92	0.597	79–88	12.16 (0.10)a
Total	478			89–98	12.56 (0.10)b
				99–08	12.66 (0.10)b
Precipitation					
Decade	3	1.04	0.37	69–78	38.96 (3.93)a
Decade × month	33	0.55	0.98	79–88	38.15 (3.93)a
Total	475			89–98	31.06 (4.00)a
				99–08	40.00 (3.93)a

Table 3 (continued)

Source of variation	df	F	P	Least square means (SE) by decade	
<i>Last Post</i>					
Maximum temperature					
Decade	3	11.84	<0.001	69–78	26.72 (0.14)a
Decade × month	33	1.84	0.70	79–88	27.39 (0.14)b
Total	431			89–98	27.55 (0.15)bc
				99–08	27.85 (0.13)bd
Minimum temperature					
Decade	3	7.41	<0.001	69–78	12.07 (0.10)a
Decade × month	33	0.985	0.50	79–88	12.18 (0.10)b
Total	455			89–98	12.45 (0.10)c
				99–08	12.67 (0.10)cb
Precipitation					
Decade	3	2.02	0.11	69–78	46.38 (3.67)a
Decade × month	33	0.81	0.77	79–88	36.42 (3.67)a
Total	478			89–98	37.43 (3.69)a
				99–08	37.50 (3.67)a

(Fig. 1 and Table 1). Sites were chosen to represent a range of tree mortality from very high to low.

2.2. Assessments of symptoms and mortality

At each site, nine linear belt transects (50 m × 100 m) were established. Locations of transects were recorded using a Global Positioning System (GPS; Garmin 60 series, Johannesburg, RSA). Within each transect, *E. ingens* trees were tagged using the GPS. The diameters of the tagged trees were measured at breast height (approximately 1.4 m; DBH). In each transect, numbers of juvenile (no fruit production) and adult trees (fruit production), and dead and live trees were recorded. Damage by beetles, moths and vertebrates were scored separately as binary variables (present/absent). Gray discoloration (a symptom clearly associated with disease and mortality) was also scored based on a ranking system: (1) primary tier branches discolored, (2) primary and secondary tier branches discolored, and (3) primary, secondary, and tertiary tier branches discolored, with primary branches representing the lowest, oldest branches.

2.3. Weather data

Monthly precipitation and temperature (minimum and maximum) data were obtained from 1969 to 2008 (Enzelsberg, Wolfaan, Capricorn and Last Post) and 1996 to 2008 (Euphorbia drive and Homestead) from the South African Weather Service (www.weathersa.co.za) (Table 1). Weather stations were selected that were the closest possible while providing the type of weather data needed for analyses.

2.4. Data analyses

Differences in mortality among sites and the main symptoms associated with *E. ingens* decline (graying) were analyzed using Kruskal–Wallis one-way ANOVA on ranks using

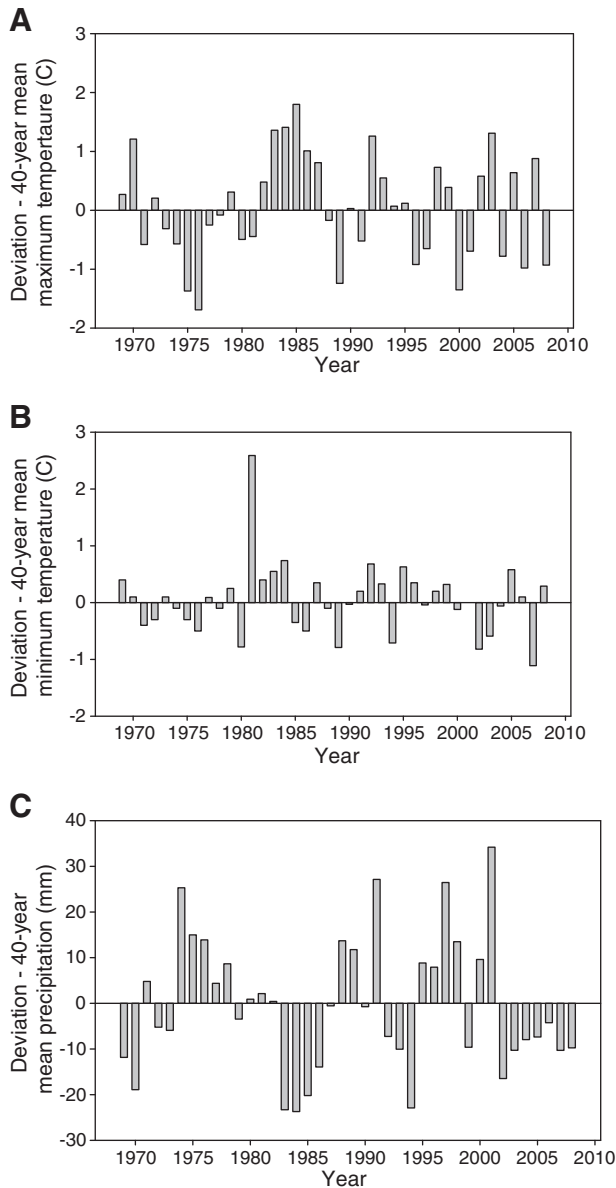


Fig. 2. Deviations from the 40 year mean for A) mean annual maximum temperature, B) mean annual minimum temperature, and C) mean annual precipitation for the period from 1969 to 2008 at the at the Enzelsberg study site, South Africa.

medians for independent variables (moth damage, bird damage, insect damage, basal damage, gray discoloration, spots, mature and juvenile trees) averaged by transect ($n=9$ per site). Principal components analysis (PCA) was used to visually detect potential relationships among the three symptoms observed to be most commonly associated with decline (graying, spots and moth damage) and weather variables (monthly minimum and maximum temperatures, and monthly precipitation). Weather data for PCA was partitioned by season (summer: December, January, February; autumn: March, April, May; winter: June, July, August; spring: September, October, November) for the four decades

preceding the establishment of transects (Capricorn, Last Post, Enzelsberg and Wolfaan) or the last decade preceding establishment (Euphorbia drive/Homestead for which earlier temperature data were not available).

To determine whether temperature and/or precipitation varied by decade (Capricorn, Last Post, Enzelsberg and Wolfaan), we used two-way ANOVAs with site, decade and month as potential sources of variation and minimum or maximum monthly temperatures or monthly precipitation as predictor variables. For Capricorn and Last Post, maximum and minimum temperature and precipitation data were normal and possessed equal variances and ANOVAs were conducted on raw data. For Enzelsberg and Wolfaan, maximum and minimum temperatures and precipitations, respectively, failed to meet assumptions of normality. Transforming ($\ln + 1$; to account for zeros in the data), failed to normalize these data. Likewise, for all four sites, precipitation data were not normal, nor did they exhibit equal variances. While data in these cases violated some assumptions of ANOVA, the large sample size allowed the use of this more robust test. Therefore, two-way ANOVAs were conducted on transformed or raw data depending on their distributions. Mean separations for one-way ANOVAs were conducted using the Tukey Test. Mean separations for two-way ANOVAs were conducted using the Holm–Sidak method. All analyses, except PCA, were conducted using SigmaStat within SigmaPlot 11.2 (Systat Software Inc., San Jose, CA). PCAs were conducted using the Community Analysis Package (CAP; Pisces Conservation Ltd., Lymington, UK).

To investigate whether temperature or precipitation has changed over time at the study sites, in ways that may have influenced the die-off of *E. ingens*, 40 year means for maximum and minimum temperatures and precipitations were calculated for the four sites for which long-term weather data were available (Capricorn, Last Post, Enzelsberg, and Wolfaan) or the last decade for Euphorbia drive/Homestead. Annual deviations from these means were then calculated and plotted over time. An index of water balance (WB) for each of the sites was also calculated. This index is based on the difference between precipitation and potential evapotranspiration (PET) and was included to provide information on how changes in WB in these systems may relate to the die-off. The index is calculated using monthly means of daily temperature, total monthly precipitation and day length adjusted for latitude. Its derivation is fully described in [Thornthwaite \(1948\)](#). Deviations from the mean WB index over time were calculated and plotted to determine whether WB at the sites was becoming more negative and whether there is a threshold water deficit that may help predict the onset of mortality at a site.

3. Results

3.1. Variables associated with *E. ingens* die-off

Table 2 summarizes mortality, percentage of juvenile vs adult trees, and symptoms for each study site. Graying, spots and moth damage appeared to be associated with die-off of *E. ingens* and these were included in further analyses.

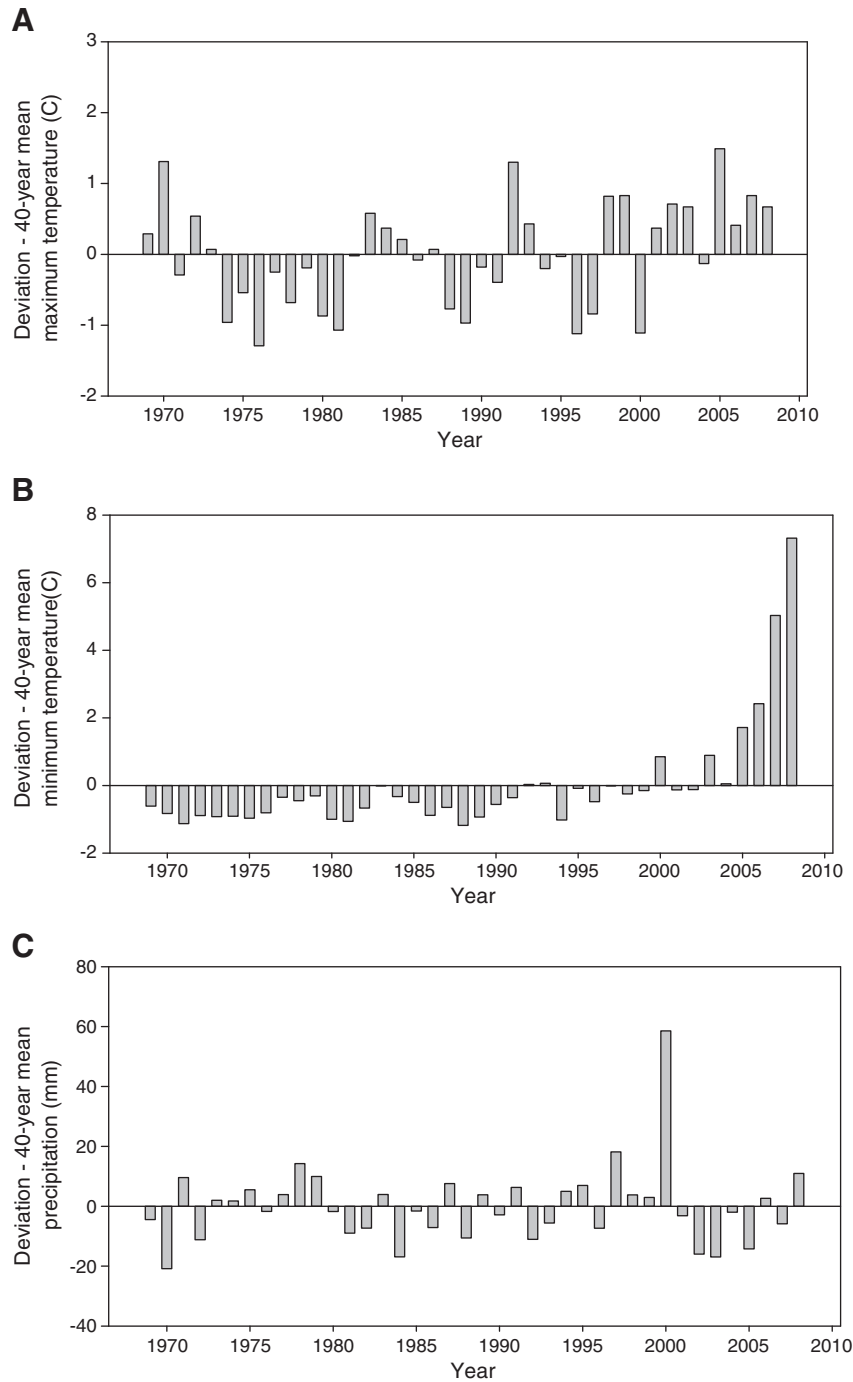


Fig. 3. Deviations from the 40 year mean for A) mean annual maximum temperature, B) mean annual minimum temperature, and C) mean annual precipitation for the period from 1969 to 2008 at the at the Wolfaan study site, South Africa.

Mortality of *E. ingens* varied significantly among sites ($H=21.560$, $df=5$, $P<0.001$). The mean proportion of dead trees was highest at the adjacent Homestead and Euphorbia drive sites ($\bar{x}=0.307$, $SE=0.120$ and $\bar{x}=0.235$, $SE=0.112$, respectively) followed by Enzelsberg ($\bar{x}=0.137$, $SE=0.049$). The mean proportion of dead trees was low at Last Post and Wolfaan ($\bar{x}=0.026$, $SE=0.015$ and $\bar{x}=0.050$, $SE=0.028$,

respectively) and almost non-existent at Capricorn ($\bar{x}=0.008$, $SE=0.008$). The proportion of juvenile trees varied between sites from the North West and Limpopo Province ($H=41.590$, $df=5$, $P<0.001$). The highest proportion of juvenile trees were found at the two healthier sites in the North West Province compared to much lower levels at the more diseased sites in the Limpopo Province (Table 2).

The degree of graying present on trees was significantly different among sites ($H=43.670$, $df=5$, $P<0.001$). The mean ranking of graying (measured on a scale from 0 (none)–3 (high)) was highest at Euphorbia drive ($\bar{x}=1.890$, $SE=0.058$), followed by Last Post ($\bar{x}=1.330$, $SE=0.840$) and Homestead ($\bar{x}=1.240$, $SE=0.110$). Graying was intermediate at Capricorn ($\bar{x}=0.549$, $SE=0.152$) and least apparent on trees at Wolfaan ($\bar{x}=0.220$, $SE=0.090$) and Enzelsberg ($\bar{x}=0.030$, $SE=0.010$).

Moth damage was found to be significantly different between sites ($H=29.210$, $df=5$, $P<0.001$). Trees with the highest proportion of moth damage were found at the two sites in Mokopane, Euphorbia drive ($\bar{x}=0.889$, $SE=0.111$) and Homestead ($\bar{x}=0.733$, $SE=0.073$). Moth damage was also relatively high at Enzelsberg ($\bar{x}=0.652$, $SE=0.076$) and Last Post ($\bar{x}=0.616$, $SE=0.033$). Low levels of moth damage were found to occur at Capricorn ($\bar{x}=0.431$, $SE=0.063$) and Wolfaan ($\bar{x}=0.122$, $SE=0.060$).

White spots (diameter of 1–2 mm) on the green branches were found to be present at high levels at all of the sites ($H=23.500$, $df=5$, $P=0.003$), with the highest occurrences being at Euphorbia drive ($\bar{x}=1.000$, $SE=0.000$) and Last Post ($\bar{x}=0.981$, $SE=0.009$). High levels of spots were also found at Homestead ($\bar{x}=0.940$, $SE=0.026$) followed by Enzelsberg ($\bar{x}=0.782$, $SE=0.048$) and Wolfaan ($\bar{x}=0.761$, $SE=0.082$).

3.2. Relationships between climate and *E. ingens* die-off

Results of ANOVAs comparing monthly means for maximum and minimum temperatures and precipitations among decades (decade 1=1969–1978, decade 2=1979–1988, decade 3=1989–1998, decade 4=1999–2008) for each site (except Euphorbia drive/Homestead for which long-term data were missing for temperature) indicated significant differences in environmental conditions between the sites for these periods (Table 3). Precipitation showed an overall decline, or increased inter-annual variability, at each site over the 40 year time period that was analyzed (Figs. 2–6).

For the Euphorbia drive/Homestead sites, deviations from the 10-year-mean showed a clear trend in warming for maximum temperatures (Fig. 4). Deviations from the 10-year-mean for minimum temperatures were much more variable and showed no clear trend. For the Capricorn and Last Post sites (Figs. 5 and 6), there were clear trends of increasing maximum and minimum temperatures over the 40 year period. At Last Post, trends in temperature closely mirrored those at Capricorn. For Wolfaan (Fig. 3) decade four exhibited significant warming from decades one, two and three and these differences were due to increased minimum temperatures in May through August. Enzelsberg (Fig. 2) differed from all other sites in that there was no consistent

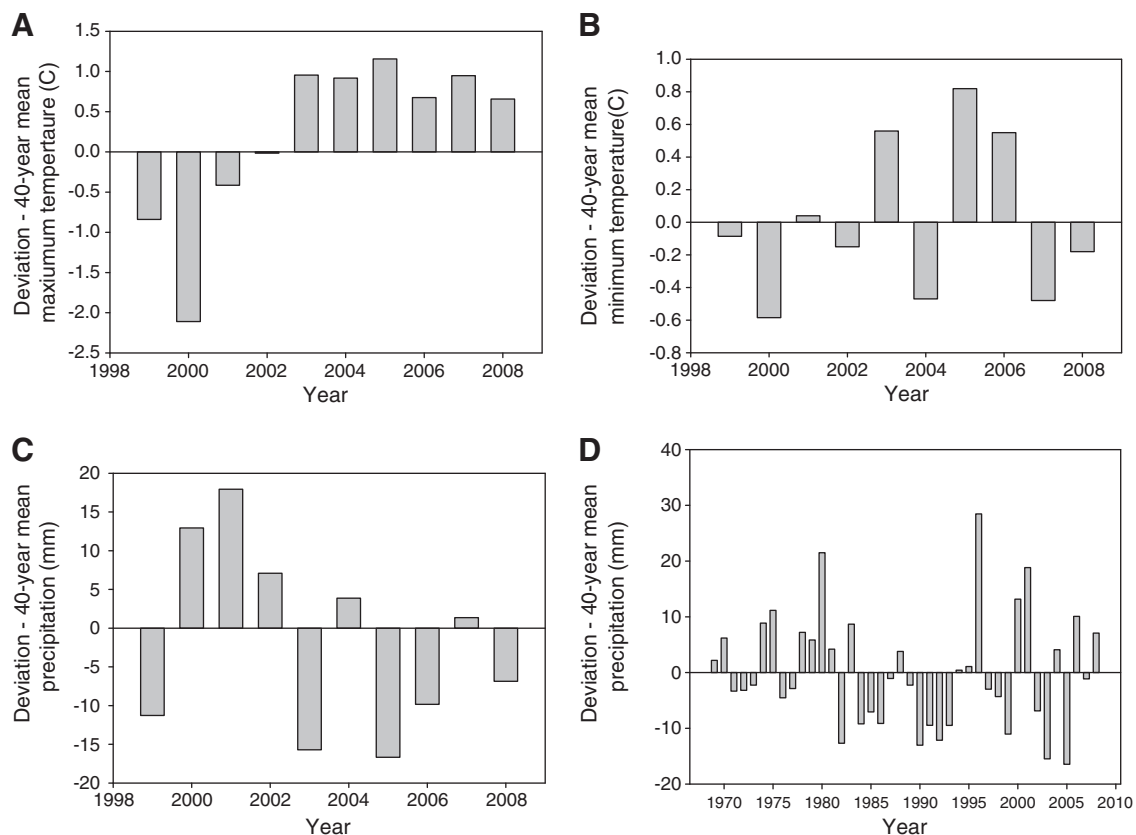


Fig. 4. Deviations from the 10 year mean for A) mean annual maximum temperature, B) mean annual minimum temperature, and C) mean annual precipitation for the period from 1999 to 2008, and for D) deviations from the 40 year mean for mean annual precipitation for the period from 1969 to 2008 at the Euphorbia drive/Homestead study sites, South Africa.

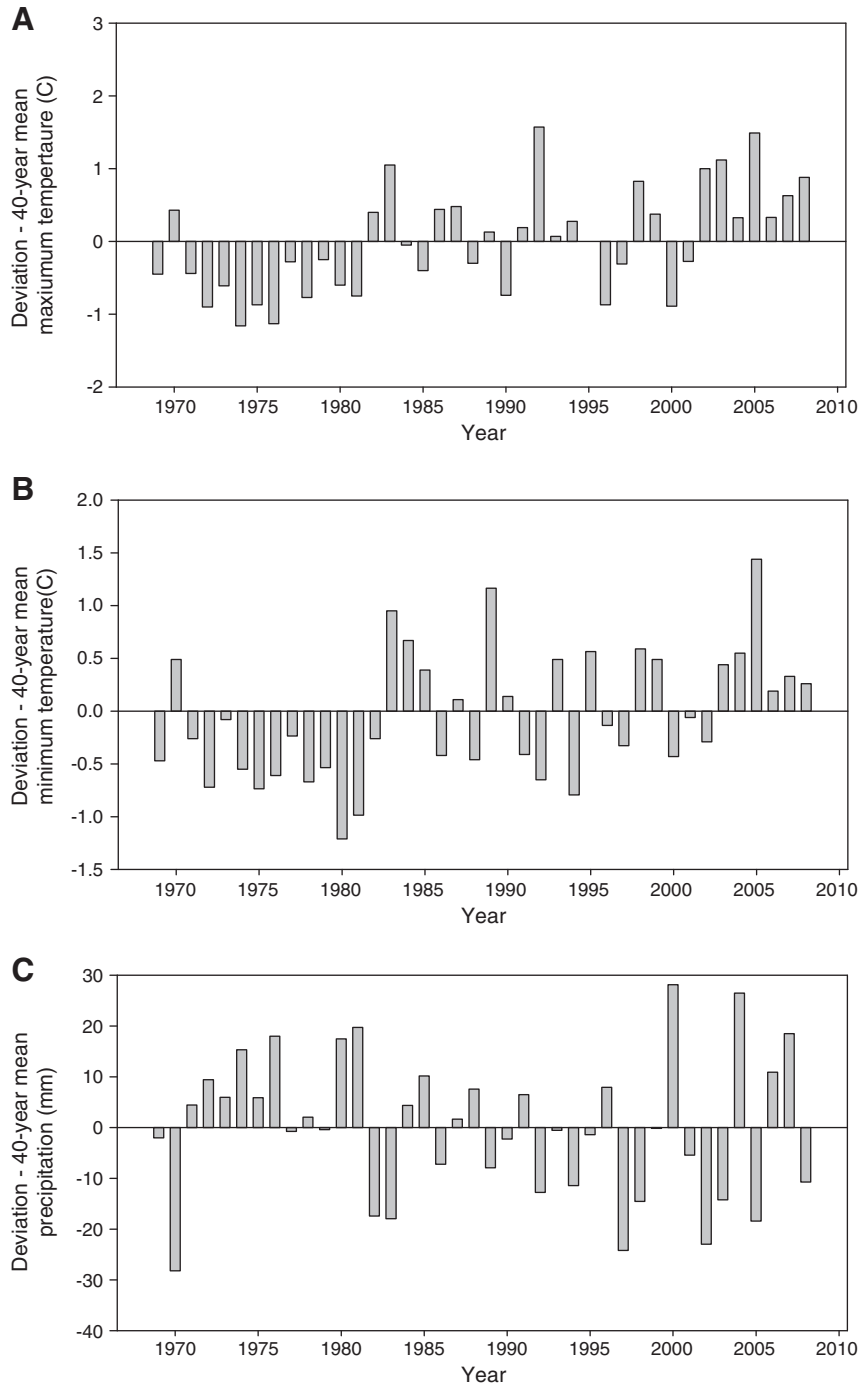


Fig. 5. Deviations from the 40 year mean for A) mean annual maximum temperature, B) mean annual minimum temperature, and C) mean annual precipitation for the period from 1969 to 2008 at the at the Capricorn study site, South Africa.

increase in maximum or minimum temperatures over the 40 year period.

Euphorbia drive/Homestead, by far, exhibited the greatest (most negative value) mean PET followed by Enzelsberg, Last Post, Capricorn, and Wolfaan (Table 4). Estimates of WB indicated a greater moisture deficit (more negative values) at sites with high levels of symptoms and mortality (Euphorbia drive/

Homestead, Capricorn and Last Post) than at the two relatively healthy sites (Enzelsberg and Wolfaan) (Figs. 7 and 8, Table 4).

3.3. Principal components analyses

PCAs were run using data for branch graying, spots on the branches, moth damage and weather data for each decade for

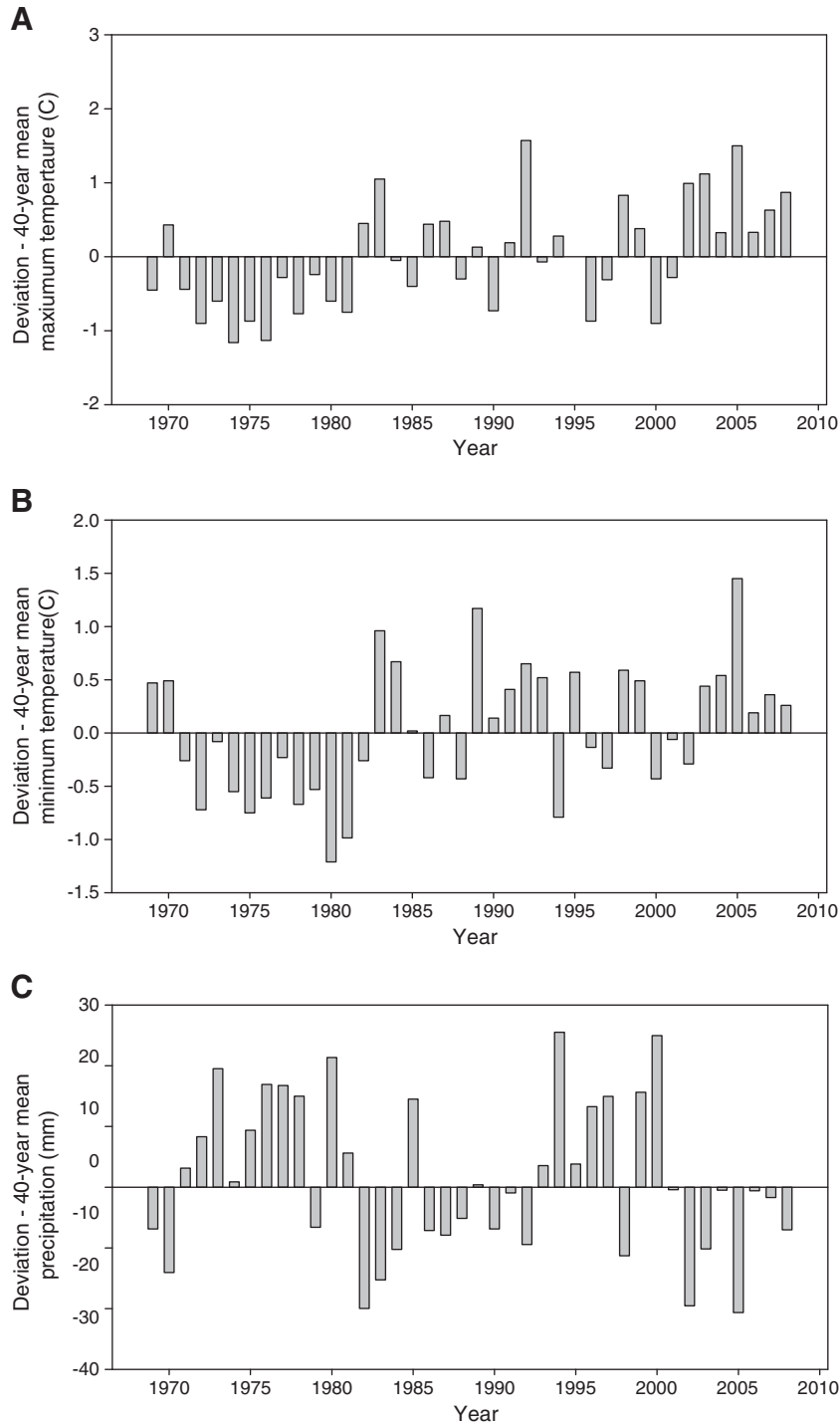


Fig. 6. Deviations from the 40 year mean for A) mean annual maximum temperature, B) mean annual minimum temperature, and C) mean annual precipitation for the period from 1969 to 2008 at the the Last Post study site, South Africa.

Last Post, Capricorn, Enzelsberg, and Wolfaan, and for the last decade for Euphorbia drive/Homestead. PCA plots were done for all sites except Euphorbia drive/Homestead in decade one (1969–1978) and two (1979–1988). These two decades were chosen because climatic influences on the die-off may have begun in the decade(s) immediately preceding the development

of obvious symptoms and widespread mortality. In the ordination plot for the fourth decade (1999–2008) (including all sites), sites with the highest tree die-off cluster with spots, moth damage, graying, higher maximum temperatures and lower precipitation, particularly in the dry winter and fall periods while the healthier sites cluster with higher precipitation and lower temperatures.

Table 4

Mean (SE) maximum and minimum temperatures (°C), precipitations (cm), potential evapotranspiration (PET) and water balance (WB) (PET minus precipitation) at six study sites exhibiting various levels of die-off of *Euphorbia ingens* in South Africa.

Site	Decade	Max. temperature	Min. temperature	Precipitation	PET	WB
Enzelsberg	1969–1978	27.0 (0.3)	11.7 (0.1)	63.4 (5.1)	–94.7 (2.0)	–30.8 (6.5)
	1979–1988	28.0 (0.3)	12.1 (0.3)	51.6 (4.8)	–98.2 (1.6)	–46.3 (6.4)
	1989–1998	27.3 (0.3)	11.9 (0.2)	66.3 (6.0)	–96.7 (0.9)	–29.8 (6.5)
	1999–2008	27.3 (0.3)	11.7 (0.2)	55.9 (5.6)	–95.5 (1.3)	–38.2 (6.6)
	Total period	27.4 (0.1)	11.8 (0.1)	54.3 (2.8)	–96.2 (0.8)	–36.3 (3.3)
Wolfaan	1969–1978	26.1 (0.2)	11.1 (0.1)	54.9 (3.2)	–90.8 (0.8)	–35.9 (3.5)
	1979–1988	26.2 (0.9)	11.2 (0.1)	51.7 (2.7)	–90.4 (0.8)	–38.6 (2.6)
	1989–1998	26.2 (0.3)	11.5 (0.1)	57.1 (2.6)	–89.2 (1.7)	–32.1 (3.4)
	1999–2008	26.7 (0.2)	13.7 (0.8)	56.7 (6.9)	–99.4 (3.4)	–48.2 (4.1)
	Total period	26.3 (0.1)	11.9 (0.3)	55.1 (2.1)	–92.4 (1.2)	–38.7 (1.9)
Euphorbia dr./Homestead	1969–1978	–	–	56.6 (2.5)	–	–
	1979–1988	–	–	55.6 (4.0)	–	–
	1989–1998	–	–	47.4 (2.0)	–	–
	1999–2008	27.8 (0.3)	13.6 (0.2)	55.3 (4.7)	–102.6 (1.5)	–47.3 (5.5)
	Total period	–	–	54.0 (1.8)	–	–
Capricorn	1969–1978	26.7 (0.7)	12.1 (0.2)	47.0 (4.9)	–92.2 (1.8)	–45.2 (4.9)
	1979–1988	27.4 (0.2)	12.2 (0.2)	44.7 (4.9)	–95.9 (1.5)	–51.2 (5.6)
	1989–1998	27.6 (0.3)	12.5 (0.2)	40.1 (3.2)	–96.4 (1.8)	–56.3 (3.8)
	1999–2008	27.9 (0.2)	12.7 (0.2)	45.2 (7.2)	–97.3 (1.5)	–52.1 (7.8)
	Total period	27.4 (0.1)	12.3 (0.1)	44.3 (2.6)	–95.4 (0.9)	–51.2 (2.8)
Last Post	1969–1978	26.7 (0.7)	12.1 (0.2)	55.7 (4.1)	–94.7 (2.0)	–39.0 (4.1)
	1979–1988	27.4 (0.2)	12.2 (0.2)	43.7 (4.9)	–98.2 (1.7)	–54.5 (5.9)
	1989–1998	27.6 (0.3)	12.5 (0.2)	44.9 (4.7)	–96.1 (1.1)	–51.8 (5.1)
	1999–2008	27.9 (0.2)	12.7 (0.2)	45.0 (5.4)	–96.0 (1.2)	–51.1 (5.5)
	Total period	27.4 (0.1)	12.3 (0.1)	47.3 (2.4)	–96.2 (0.8)	–49.0 (2.7)

The ordinations for the first and second decades that do not include the Euphorbia drive/Homestead site (Fig. 9A and B) mostly agree with one another and the PCA conducted for all sites for decade four (Fig. 9C). For decade two, the sites exhibiting the greatest degree of decline clustered with higher maximum and minimum temperatures, lower precipitation, graying, spots and moth damage while the relatively healthy sites clustered with lower temperatures and greater precipitation. The first two axes accounted for 84% of the variance; adding the third axis brought this estimate to 94%. For decade four, the sites exhibiting the greatest degree of die-off clustered with higher maximum temperatures, lower precipitation, graying and spots, while the relatively healthier sites clustered with lower temperatures and greater precipitation. In the ordination for decade four (Fig. 9C), the first two axes explain 88% of the variance. When the third axis was included, it was possible to account for 96% of the variance. Ordinations run for decade three (data not shown) agreed very closely with the ordination for decades one and two.

4. Discussion

The three main symptoms associated with the die-off of *E. ingens* were graying of the branches, spots on the green branches and moth damage. These symptoms varied in severity among sites, but were overall much less severe at sites in the North West Province (Wolfaan and Enzelsberg) than in the Limpopo Province. The main symptom of disease, graying of the branches, was most severe in the Limpopo Province,

particularly at the Euphorbia drive/Homestead sites, which also exhibited the highest level of mortality. Recently it has been shown that scaling and barking (similar to gray discoloration seen in this study) occur on tree-like succulent *Euphorbia* species due to prolonged exposure to the sun (Evans and Abela, 2011). This might lead to reduced photosynthesis and eventual death of branches, ultimately leading to the death of the tree.

Apart from mortality and symptoms of disease being more severe in the Limpopo Province, the presence of juvenile trees was much lower there compared to the North West Province. Baboons, giraffes, monkeys and black rhinos were present at sites in the Limpopo Province which may have affected regeneration. Higher levels of juveniles in the North West Province also could be due to more favorable climatic conditions (cooler temperatures, more stable temperatures compared to the Limpopo Province and higher rainfall), land management history and soil conditions. The influence of these factors will be investigated in the future.

The Limpopo Province sites are approximately 2° further north, and experience higher temperatures and lower rainfall, that those in the North West Province. Therefore, these sites may be marginal for *E. ingens*. If this is true, extended hot dry periods could have pushed the trees past a threshold where they are no longer able to survive. Our analyses of weather patterns over the last 40 years indicated that maximum and minimum temperatures have increased in the Limpopo Province and may be driving the die-offs. The greatest change in maximum temperatures occurred at Euphorbia drive/Homestead (with a mean 2 °C increase), the sites with the most obvious symptoms

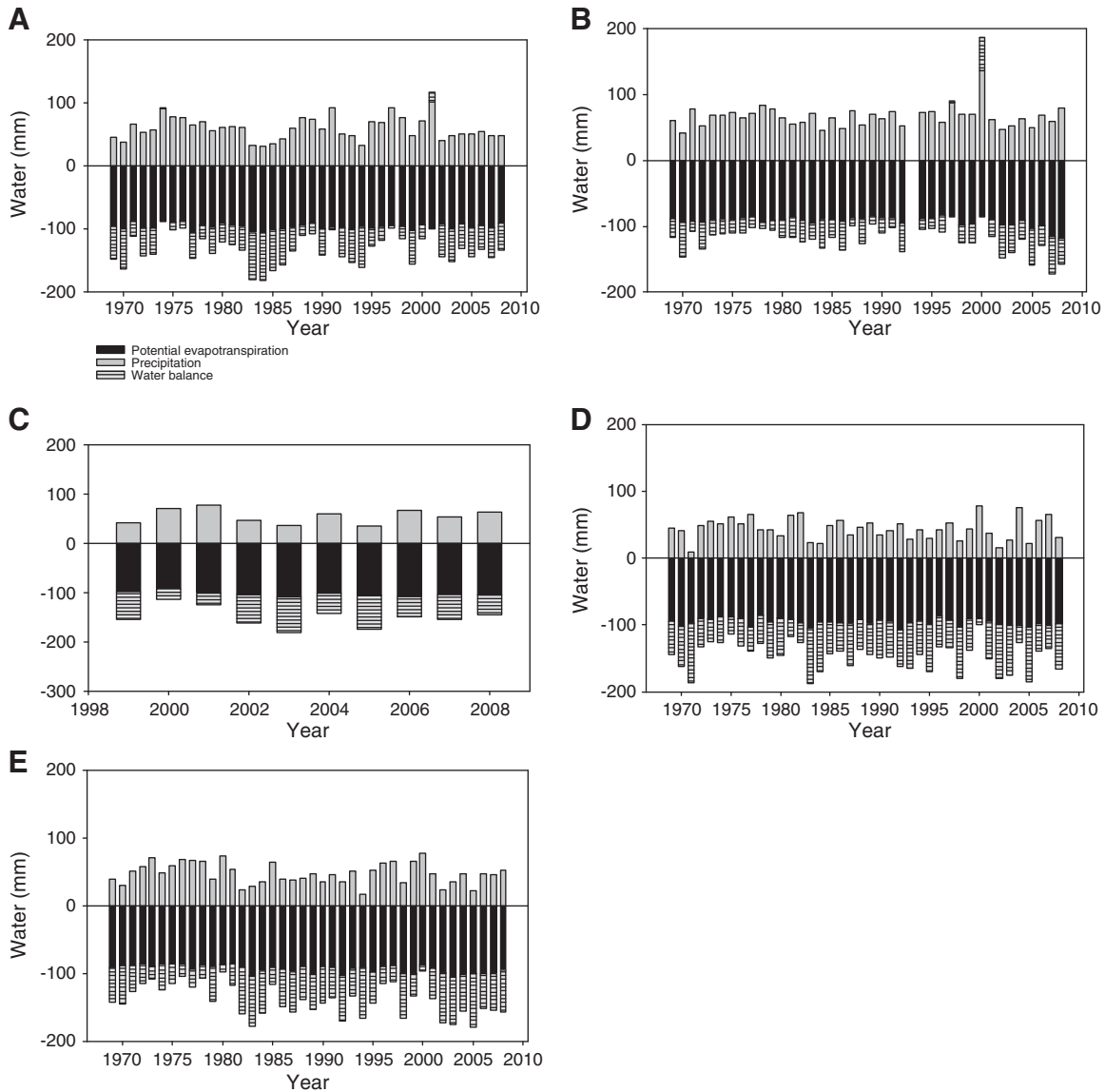


Fig. 7. Annual precipitation and estimates of potential evapotranspiration (PET) and water balance [precipitation (water input)–PET (water demand/output)] (mm) for the period from 1969 to 2008 for A) Enzelsberg, B) Wolfaan (1993 weather data was only partial so was not included), C) Euphorbia drive/Homestead (1999–2008 only), D) Capricorn, and E) Last Post study sites.

and most severe mortality. Maximum temperatures in the North West Province did not increase greatly, although Wolfaan exhibited a rise of about 0.63 °C. However, minimum temperatures at Wolfaan increased dramatically in the last decade (about 2.66 °C). Even though temperatures increased in the North West Province over the 40 year period, the mean annual maximum and minimum temperatures were still lower overall compared to those in the Limpopo Province. As temperatures increase, so does evapotranspiration. If precipitation does not increase to meet this increase in demand for moisture by plants, then stress may occur, resulting directly in mortality if stress becomes severe, or indirectly due to increased susceptibility to insects and pathogens (Dukes et al., 2009; Stone et al., 2007; Sturrock et al., 2011; Tubby and Webber, 2010). In cases where precipitation declines at the same time temperature increases,

this effect may be even more pronounced. In this study, sites with higher temperatures, higher PET and higher WB deficits exhibited the greatest degree of symptoms and mortality. While the data are not conclusive that greater water stress is the proximate cause of the die-off, they indicate that climate is playing a substantial role.

Principle component analysis indicated that the main symptoms associated with die-off (graying of stems, moth damage and spots on the green branches) were most closely associated with sites experiencing hot, dry autumn and winter periods (Fig. 9). Autumn and winter are the dry seasons for this portion of South Africa. Increases in temperature and decreases in precipitation during this period may increase stress on trees leading to greater susceptibility to insects and disease, and ultimately, mortality. Unfortunately, temperature data for

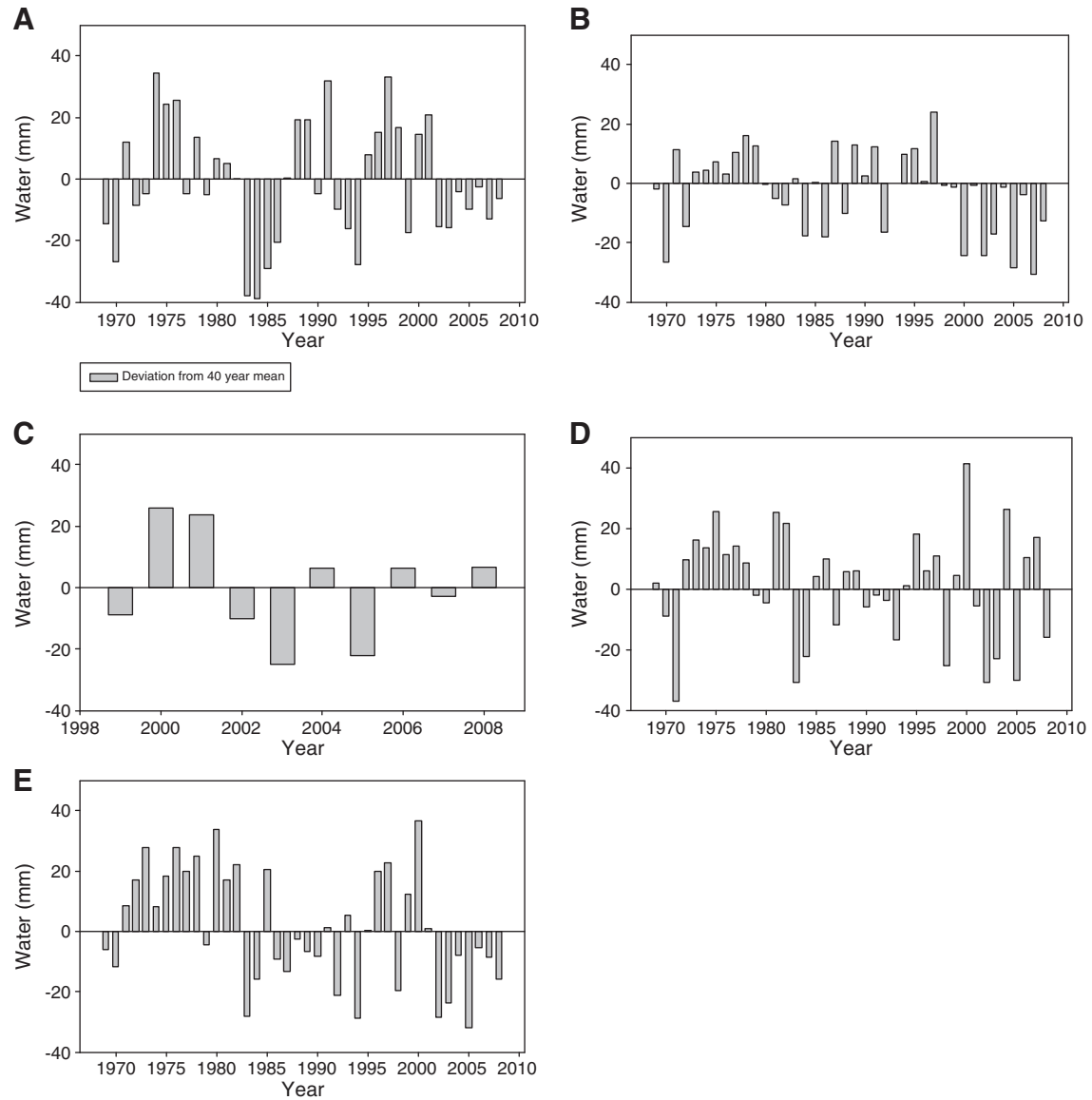


Fig. 8. Annual deviations from the 40 year mean (1969–2008) of water balance (precipitation–potential evapotranspiration, mm) estimates for A) Enzelsberg, B) Wolfaan, C) Euphorbia drive/Homestead (1999–2008 only), D) Capricorn, and E) Last Post study sites.

Euphorbia drive/Homestead, the site most severely impacted by the die-off, were available only from 1996 to 2009. Die-offs were first noticed in the mid-to-late 90s at this site, suggesting that an environmental threshold had been reached in the previous decade (1989–1998). This likely involved increased temperatures (and concurrent increased demand for water) as increases in temperature were observed at the other sites in the Limpopo Province during that period.

We were not able to definitively determine the actual cause or trigger of the *E. ingens* die-offs being observed in South Africa. However, the die-offs appear to be the result of the interaction of many factors including temperature, precipitation and various biotic components (particularly, insects and fungi). The sites analyzed also appear to occur in areas of greater

temperature increase (for the period 1950–1999) compared to other areas in the Limpopo Province (Tshiala et al., 2011). Additional work that includes a greater number of sites spanning a range of symptoms, mortality, and areas of different weather conditions and rate of climatic change will contribute to a better understanding of the links between weather conditions, climate change and die-offs of *E. ingens*.

Acknowledgments

We thank the Department of Science and Technology/National Research Foundation (DST/NRF) Centre of Excellence in Tree Health Biotechnology (CTHB) and the University of Pretoria, South Africa, for the financial support; Mark Howitt,

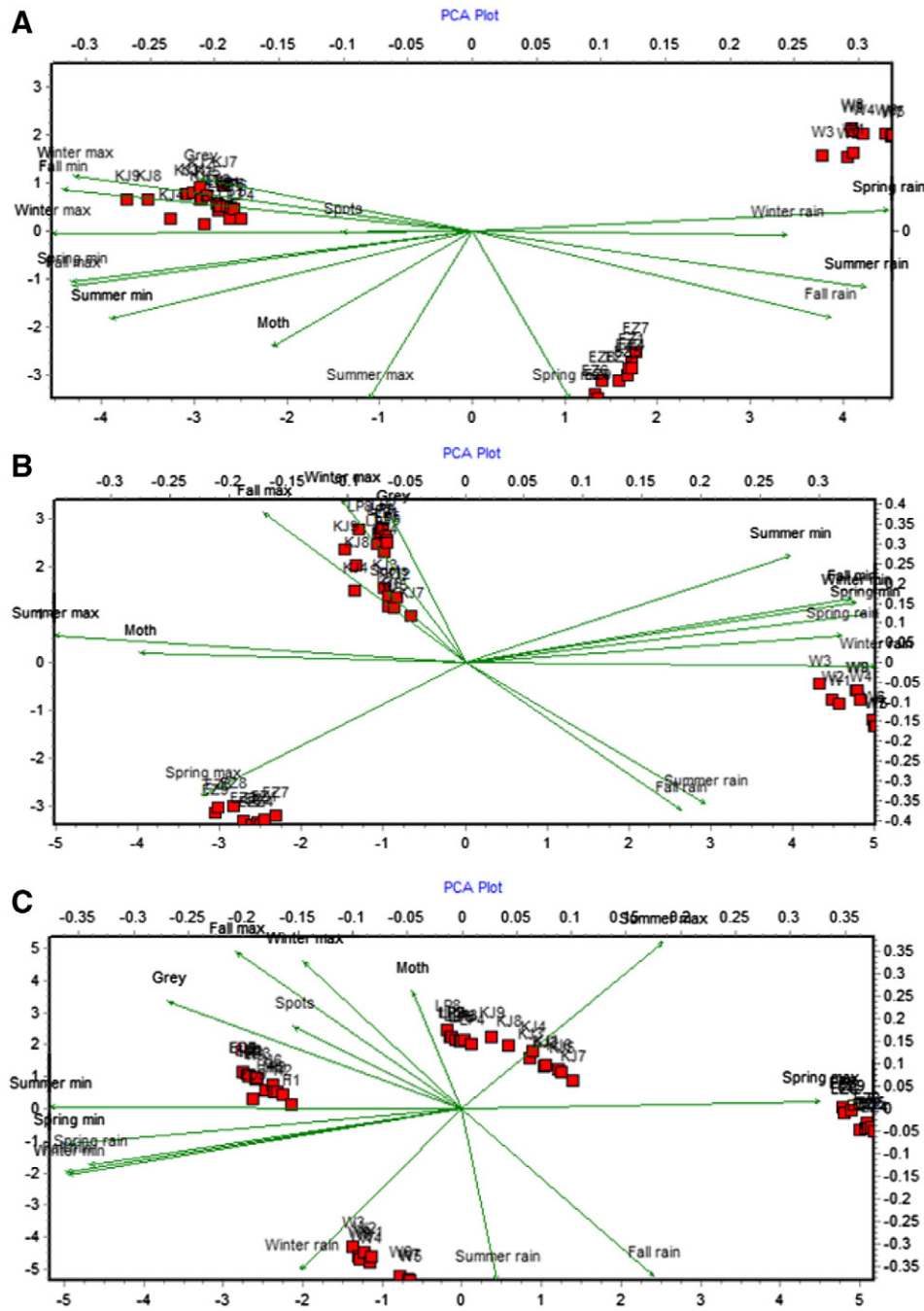


Fig. 9. PCA plots for A) for the decade from 1969 to 1978, and B) and the decade from 1979 to 1988 for the Last Post (LP), Capricorn (KJ), Enzelsberg (EZ), and Wolfaan (W) sites, and C) the decade from 1999 to 2008 for all sites [including Euphorbia drive (ED) and Homestead (H)]. Variables included were the degree of spotting and graying of *Euphorbia* trees, the presence of moth damage, mean annual maximum and minimum temperatures, and mean annual precipitation. Spotting, graying and moth damage were averaged by transect (N=9 per site).

Rentia Malan, Keith Johnson, Manie Eloff, Chris Richards, Hermien Roux and Alf Sephton are thanked for the permission to undertake field studies. We also thank the South African Weather Service for the temperature and precipitation data, the National Zoological Gardens for the permission to conduct fieldwork in Mokopane and Norbert Hahn, Soutpansberg Herbarium, for the assistance with field work.

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