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Dieback and mortality of South African fynbos shrubs is likely driven by a novel pathogen and pathogen-induced hydraulic failure

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Abstract We examined whether extensive dry season dieback and mortality in a South African fynbos community were due to drought or pathogen attack. Plant dieback and mortality have been reported elsewhere in similar plant communities suggesting potential for a widespread climatic or biotic threat to this community. We collected tissue samples from *Brunia noduliflora*, the dominant plant in the community, and cultured them for potential plant pathogens. We also measured dry season predawn and midday water potentials of healthy and stressed plants and constructed pressure-volume curves to assess turgor loss point. Plant stress and mortality were monitored over a 2-year study period. Both healthy plants and plants that displayed moderate signs of stress had dry season predawn water potentials well above their turgor loss point suggesting plants were not water stressed. However, plants displaying >60% crown dieback had much lower water potentials (as low as −12 MPa). A previously undescribed fungus (*Pythium* sp.) was isolated from the root vascular tissue of all stressed plants and was not present in healthy plants. The proximate cause of plant stress was likely pathogen-induced, while the ultimate cause of plant death appears to be extreme water stress. The present study suggests that *Brunia* (Bruniceae), *Leucadendron* (Proteaceae) and *Erica* (Ericaceae), all emblematic and dominant genera within the diverse fynbos community, may be susceptible to *Pythium* infection. This may pose a serious threat to communities already threatened by climate change.

Key words: *Brunia nodiflora*, fynbos, *Leucadendron*, Mediterranean-type ecosystem, *Pythium*, water stress.

INTRODUCTION

Dieback and death of large numbers of woody mature plants within a community are relatively rare and usually associated with extreme events, including intense and protracted drought or disease outbreaks. Drought has been linked to woody plant dieback (i.e. partial crown or canopy death) as well as whole plant death in many different types of communities, including dieback in Mediterranean-type climate region shrub communities (Peñuelas *et al.* 2001; Davis *et al.* 2002; Lloret *et al.* 2004), death and decline among trees in forests and woodlands (Ogle *et al.* 2000; Suarez *et al.* 2004; Mueller *et al.* 2005; McDowell *et al.* 2008), death of savanna trees (Albertson & Weaver 1945; Fensham *et al.* 2009), and the decline and death of desert shrubs (Miller & Huenneke 1996; Miriti *et al.* 2007). Dieback and death of plants due to drought often differentially impact co-occurring species and thus have the potential to alter community structure and/or contribute to longer-term shifts in vegetation types and community distributions (Mueller *et al.* 2005; Kelly & Goulden 2008; McDowell *et al.* 2008).

Widespread woody plant death may also be triggered by pathogens, particularly following pathogen introduction to sensitive systems or because of introduction of new biological vectors. The decline of the American chestnut (*Castanea dentata*), a former dominant of eastern North American hardwood forest, following the introduction of chestnut blight (a fungus, *Cryphonectria parasitica*) in the early 1900s is a classic example of the severe effects that pathogens can have on community structure and ecosystems (Anagnostakis 1987). Pathogens have also been linked to widespread plant dieback and death in other natural systems, including death and dieback of *Acacia* (Anderson *et al.* 2002), *Quercus* (Rizzo & Garbelotto 2003) and *Pinus* (Dobbertin *et al.* 2001).

Many regions have been affected following the introduction of *Phytophthora* to sensitive communities, including the forests of western North America (Rizzo & Garbelotto 2003), European oak forests (Brasier 1996; Robin *et al.* 1998) and the plant communities

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of Western Australia (Weste & Marks 1987; Wills 1992). In many of these regions, pathogen effects are not limited to single plant species and, in the case of the jarrah forest of Australia, up to 75% of the flora is experiencing dieback and death following the introduction of *Phytophthora* (Weste & Marks 1987). Thus, as with drought-induced death, pathogen-induced mortality of individuals and species also has the potential to heavily impact and alter native systems (Packer & Clay 2000; Wills 1992; Rizzo & Garbelotto 2003).

Globally, changes in climate and increased transport of biological organisms are likely to increase the frequency and extent of plant mortality and dieback. Global climate change is predicted to result in changes to local climatic conditions and also more erratic conditions and extreme events, including more droughts and floods (Trenberth *et al*. 2003; Dore 2005). Extensive plant mortality has been shown to be linked to both long-term drought as well as short extreme events (Allen *et al*. 2010), both of which are likely to increase in occurrence. Additionally, global connectivity and human travel networks have expanded greatly in recent years and are likely to continue to increase as the global population becomes more mobile and development spreads (The World Bank 2009). This increase in connectivity of communities also increases the likelihood of spread of pathogenic organisms to potentially sensitive areas. Drought- or pathogen-caused declines in the health, fitness and density of dominant woody species in communities are likely to have large impacts on the health of other species within affected communities and ecosystems and have potential to affect global processes as well. Understanding the causes of widespread plant declines will become increasingly important in management and mitigation of biological losses across global ecosystems.

The Bruniaceae family is endemic to South Africa and the genus *Brunia* is endemic to the Western Cape. This genus is one of the emblematic genera of the fynbos shrub community, which dominates the Mediterranean-type climate region of South Africa. As such, there has been interest in both its evolutionary history and structural characteristics and these have been examined in several previous studies (Carlquist 1978, 1991; Quint & Classen-Bockhoff 2006).

In 2009, we observed dead and dying shrubs in a fynbos shrub community dominated by *Brunia noduliflora* Goldblatt & J.C. Manning (Bruniaceae) within the Paarl Mountain Nature Reserve, Western Cape, South Africa. We examined whether plant mortality in this community was likely caused by abiotic (i.e. drought) or biotic (i.e. pathogen) factors. If drought was the primary factor affecting plant dieback, we hypothesized that plant water status would be related to plants displaying signs of stress and that plants within the community would have water potentials that were indicative of water stress. If a pathogen was responsible for the dieback and mortality of plants, we hypothesized that plants with signs of stress and dieback would contain pathogenic microorganisms that were absent in healthy individuals and which were likely to cause the observed symptoms in sick plants.

METHODS

Location

During the dry season, in March 2009, dieback was observed in a population of *B. noduliflora* (=*B. nodiflora*; Goldblatt & Manning 2000; Germishuizen *et al*. 2006) within the Paarl Mountain Nature Reserve, Western Cape, South Africa. A 20 m by 40 m site was established in the dieback area on a Southeast facing slope (S 33°43.409 E 18°55.726 at 575 m elevation).

The site receives approximately 850 mm of rain annually, with little rain falling during the summer months as is typical of the Mediterranean-type climate of the region (Appendix S1). However, the year that the study site was established, the summer had been drier than average. In summer 2009, this site received 16 mm of rainfall from January to March (1 mm rain in January, 10 mm in February and 5 mm in March). This value is much lower than occurs in most years when precipitation typically totals 75 mm for these months. The months of January to May 2009 all exhibited below average rainfall. Many plants at the site appeared to be quite dry and dieback was observed in *B. noduliflora* individuals at this site in March 2009 near the end of the summer dry season.

The area we sampled burned in March 2008 and was dominated by *B. noduliflora* that were resprouting from basal lignotubers and had shoots that were 1 and 2 years old during the period of the present study. Some individuals in the stand likely had been killed by the fire and were not resprouting, but most of the *B. noduliflora* individuals (>90%) in the stand had vigorously resprouted following fire. This is similar to the fire survival rate reported previously for this species (Le Maitre *et al*. 1992). The burned area was rather extensive and extended well beyond the area in which the study site was established.

Extent of dieback

The study site was established in a patch of *B. noduliflora* plants that exhibited varying degrees of stress or dieback. The patch in which the study site was established was surrounded by a much larger area that was also dominated by *B. noduliflora* but which displayed only minimal signs of stress. Plants within the study site were categorized as being healthy (all branches alive and all leaves green), stressed (all branches alive but leaves yellow or red) or having dieback (some branches dead) (Appendix S2). The circumference and diameter (measured in two perpendicular directions) of plant bases, number of shoots and height of the *B. noduliflora* individuals within the site were determined. Basal measurements were used to calculate the two-dimensional area of

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plant lignotubers at ground height. For plants with dieback, the number of branches alive and the number of branches dead were counted and used to determine the percentage dieback as the number of dead branches divided by the total number of branches (number of dead plus the number of alive branches).

The area around the established site was surveyed to determine the approximate area containing plants showing dieback and signs of stress. These measurements and observations were conducted on plants within the site in March 2009. The site was resurveyed in January 2010 to determine whether the condition of plants at the site had changed. Additionally, in 2010, co-occurring individuals of *Leucadendron salignum* and *Erica* spp. were surveyed and the % mortality in these species determined.

Pressure-volume analysis

Branches were collected from six healthy individuals of *B. noduliflora* in the field in March 2009, placed in sealed plastic bags and transported on ice to the laboratory at Stellenbosch University to construct pressure-volume curves. Plants were hydrated in a laboratory by cutting off the cut ends of branches under water to remove air emboli. Branches were then allowed to sit with their cut ends in water while the leaves were bagged for about 2 h. Branchlets were removed from six unique branches and were weighed, the water potential was measured with a pressure chamber (Model 2001, Plant Moisture Stress, Corvalis, OR, USA), and then branchlets were re-weighed. The saturated weight was calculated by averaging the weight before and after the pressure chamber measurement. Branchlets were bagged or left on a bench top to dry and their weight and water potentials were measured periodically. After the experiment, the branchlets were dried in a drying oven at 60°C for about 7 days to obtain dry weight. The relative water content was calculated as weight measured throughout the dry-down minus dry weight divided by saturated weight minus dry weight. Pressure-volume curves were constructed and analysed following Koide *et al.* (1989) and were used to calculate turgor loss point.

Water status

Water potentials were measured on branchlets from eight healthy and eight stressed individuals at predawn and midday in the field using a pressure chamber (Model 2000 Pressure Chamber Instrument, PMS Instruments, Corvalis, Oregon, USA). Measures were made on the same plants at predawn and midday on the same day during the dry season in March 2009 and all water potential samples were from upper canopy and fully illuminated branchlets. Midday samples were collected between 12.00 and 13.00 hours on a sunny day and were measured immediately in the field.

Additionally, water potentials were measured at predawn on 34 randomly selected individuals from within the site on a single day in March 2009. Only the living branches of plants that displayed dieback were sampled. These data were used to analyse the relationship between water status and percentage dieback across the site.

Microorganism isolation

Leaves, stems and roots were collected from three healthy individuals and from three stressed plants from an area adjacent to the study site in March 2009. Five stem and root sections of approximately 20 mm in length and 8 mm in diameter were collected from each plant. Samples were collected in the field, placed in separate sealed plastic bags and transported to the laboratory at Stellenbosch University for analysis. No individuals with dieback were sampled to reduce the risk of detection of opportunistic species that may attack already diseased or dead tissues. Additional samples from six individuals were collected in March 2010.

Collected samples were surface sterilized by submerging samples in 70% ethanol for 30 s, 1% sodium hypochlorite for 3 min, and washed in sterile ddH_2O for 15 min. These stems were cut into approximately 2.5 by 2.5 mm sections under sterile conditions. Half of the sections were placed on Petri dishes containing 2% malt extract agar (MEA, Biolab, Midrand, South Africa) and water agar. The other half of the sections were placed on selective media used to isolate pythiaceous fungi from roots and soil. These included PARPH medium (Kannwischer & Mitchell 1978) for isolation of *Phytophthora*, and PARP medium for isolation of *Pythium* (PARPH medium excluding hymexazol). Plates were incubated at 25°C for 2 weeks in the dark. Fungal colonies were removed as they appeared and plated onto separate dishes containing their respective media. Plates were discarded when no new colonies appeared. Fungal taxa were grouped into morphotypes based on colony and microscopic characters.

In addition, soil samples were collected during the summer of 2010 from the rhizosphere of four stressed individuals. Approximately 250 g of soil was sampled at a depth of 5–20 cm. Samples were sent to the Forestry and Agricultural Biotechnology Institute at the University of Pretoria, South Africa for fungal isolations. Each soil sample was baited with *Citrus* leaf discs following methods of Grimm and Alexander (1973) and leaf disks were plated onto PARP and PARPH medium. Plates were incubated at 25°C for 2 weeks in the dark and monitored for the growth of pythiaceous fungi.

Analysis

Linear regression models were used to examine the relationships between plant traits, including height, basal area, minimum branch diameter of burned branches and number of shoots, with the percentage canopy dieback displayed by each plant. Those sampled plant traits were also compared among healthy, stressed and dieback groups using ANOVAs. Paired *t*-tests were used to test for differences within treatments at predawn and midday and *t*-tests were used to test for differences among treatments at predawn and among treatments at midday. Differences between predawn and midday water potentials and turgor loss point were tested using ANOVAs followed by Dunnett *post-hoc* analyses comparing water potentials with the turgor loss point. Statistical analyses were performed using Minitab (Release 14.12.0, Minitab Inc., State College, PA, USA) and Statview (v. 5.0.1, SAS Institute Inc., Cary, NC, USA). Alpha was set at 0.05 for all comparisons.

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RESULTS

Extent of plant dieback and death

In 2009, the area of dieback was restricted to a patch of approximately 60 m by 180 m. This area was dominated by *B. noduliflora*; however, the extent of *B. noduliflora* at this site extended well beyond the area that contained stressed and dead individuals. Among the *B. noduliflora* that had sprouted following fire the previous year, 14.6% of the plants appeared healthy, 29.2% showed signs of stress (yellowing and red leaves), 49.9% had dieback (some dead branches) and 6.3% appeared dead (had no living branches) (Appendix S2). Plants with dieback had an average of 39.8% dead branches within their canopies.

In 2010, the area of dieback had increased to a patch of approximately 64 m by 260 m. Much of this area of dieback had increased in the direction parallel to a foot path that extended adjacent to the study area. There was an additional patch of dieback several hundred metres downslope of the foot path in an area that had burned in March 2009 and contained resprouting *B. noduliflora* plants.

Among the *B. noduliflora* in the sampled area, the number of dead plants greatly increased in 2010 compared with 2009, up to 23.3% of the plants in the study area showing complete crown death compared with 6.3% with complete crown death the previous year. In 2010, 15.5% of the plants appeared healthy, 34.1% showed signs of stress including yellowing and red leaves, 18.8% had dieback and 23.3% had no living branches.

Individuals of *L. salignum* P.J. Bergius (Proteaceae) and two *Erica* spp. (Ericaceae) were sampled in 2010. These species represented the other resprouting shrub species that occurred at this site, but they were relatively rare in the sampled area. In 2010 25% of *L. salignum* individuals displayed dieback or were dead ($n = 12$) and 38.5% and 42.8% of each of the two common *Erica* spp. displayed dieback or death ($n = 13$ and 21 respectively). Some stress and dieback had been recorded among these species in 2009 but it had not been quantified.

Forty-four individuals of *B. noduliflora* at the site were surveyed and tagged in 2009 and re-sampled again about 1 year later in 2010. Among plants that were stressed or had dieback in 2009, there was no evidence of recovery in 2010 and many plants that had previously displayed only minor levels of stress or dieback or that previously appeared healthy had declined in their health. Many plants that had previously displayed partial canopy dieback displayed full canopy death in 2010. Among the plants scored as healthy in 2009, 28.6% appeared stressed in the 2010 survey. Among the plants that were stressed in 2009, 82.3% still appeared stressed in 2010 and 11.7% now displayed dieback. For plants with dieback in 2009, 63.2% displayed complete crown death in 2010 and most of the remaining plants (26.3%) had increased in their level of crown dieback. Plants that had full canopy death in 2009 showed no signs of resprouting in 2010, confirming that whole crown dieback was indicative of plant death.

Individuals of *B. noduliflora* at the site had a mean crown height of 0.54 ± 0.02 m and a mean basal area of 0.35 ± 0.49 m^2. The mean total number of shoots among individuals (including both living and dead branches for plants with dieback) was 48.7 ± 3.6. Minimum branch diameter of burnt shoots, an estimate of fire intensity, was 1.6 ± 0.1 mm. None of these parameters was different among healthy, stressed or dieback plants and there was no correlation among these parameters and percentage of canopy dieback (ANOVAs and linear regressions, $n = 44$, $P > 0.05$ for all). This suggests no relationships among plant health and plant size/age and fire intensity.

Water status

Healthy and stressed plants had similar access to soil water, but differed in their midday water use. Healthy plants (all leaves green) and stressed plants (some leaves yellow or red) had water potentials that were not different at predawn (Fig. 1; -0.63 ± 0.03 MPa for healthy plants and -0.75 ± 0.08 MPa for stressed

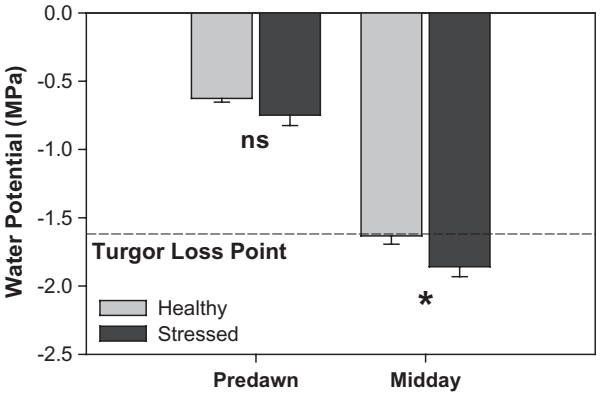

Fig. 1. Dry season water potential at predawn and midday of healthy *Brunia noduliflora* plants (grey bars; $n = 8$) and plants that appeared stressed (black bars; $n = 8$). Water potentials were not different between healthy and stressed plants at predawn ($P = 0.167$), but were significantly different among healthy and stressed plants at midday ($P = 0.033$; as indicated by the asterisk). Both healthy and stressed plants displayed a significant reduction in their water potential from predawn to midday ($P < 0.001$ for both). The turgor loss point of healthy *B. noduliflora* at this site is shown as the dashed line (−1.62 MPa). ns, not significant.

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plants; $t = 1.52$, $n = 8$, $P = 0.167$). At midday, stressed plants had water potentials that were significantly more negative than healthy plants (Fig. 1; -1.63 ± 0.06 MPa for healthy plants and -1.86 ± 0.07 MPa for stressed plants; $t = 2.38$, $n = 8$, $P = 0.033$).

Both healthy and stressed plants had water potentials that indicated that they have access to soil moisture and have water potentials that indicate that they could still expand tissues and grow. The turgor loss point for healthy *B. noduliflora* at this site was -1.62 ± 0.06 MPa, which is significantly more negative than the predawn water potentials of stressed and healthy plants (ANOVA, $P < 0.001$; Dunnett *post-hoc* test, $P < 0.001$ for both healthy and stressed plants for comparison of treatment water potentials with turgor loss point). At midday, healthy plants had water potentials that were not different from the turgor loss point, but stressed plants had water potentials that were significantly lower than the measured turgor loss point (ANOVA, $P = 0.030$; Dunnett *post-hoc* test, $P = 0.993$ for healthy plants and $P = 0.044$ for stressed plants).

Predawn and midday water potentials were significantly different in both healthy and stressed plants ($t = 10.06$, $n = 8$, $P < 0.001$ for healthy and $t = 10.51$, $n = 8$, $P < 0.001$ for stressed plants). Both the healthy and the stressed plants had water potentials that were approximately 1 MPa lower at midday relative to predawn (difference of 1.01 ± 0.06 MPa for healthy plants and 1.11 ± 0.11 MPa for stressed plants).

Across the site, plants with dieback (at least some branches dead) had variable water potentials; with plants with more dieback also having more negative water potentials (Fig. 2). It appears that plants remained relatively hydrated, and not different from stressed individuals, until they reached approximately 60% dieback, at which point their water potential drastically declined (Fig. 2).

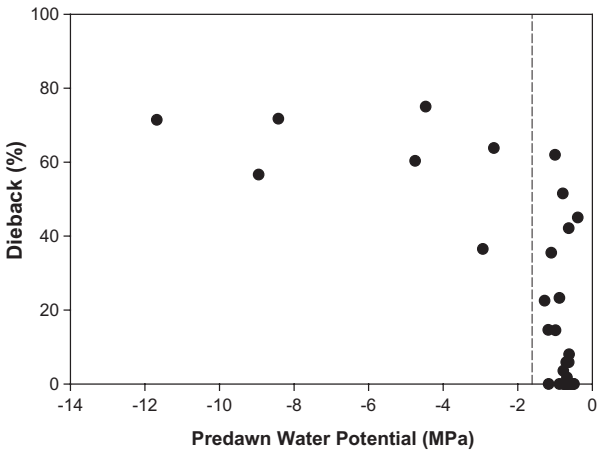

Fig. 2. Dry season water potential at predawn as a predictor of canopy dieback in *Brunia noduliflora* ($n = 34$). Each point represents one individual. The turgor loss point of *B. noduliflora* at this site is shown as the dashed line (−1.62 MPa). Plants experience up to 60% dieback while maintaining predawn water potentials above the turgor loss point. At approximately 60% canopy death, plant water potentials greatly decline.

Microorganism isolation

In total, 37 fungal isolates representing seven fungal morphospecies were collected from the plated 2009 samples (Table 1). Only a single morphospecies of *Pythium*, an undescribed *Pythium* sp., was frequently isolated. The other morphospecies were only occasionally isolated. The *Pythium* sp. was isolated from all of the root samples from stressed plants and never from any of the root samples from healthy plants. It was also never isolated from stems and leaves of stressed or healthy plants. Isolations from soil samples at the Forestry and Agricultural Biotechnology Institute housed at the University of Pretoria, South Africa revealed the presence of the same *Pythium* species. Samples from stressed plants collected in 2010 contained the same *Pythium* species. No isolates of other fungal taxa were obtained consistently from stressed plants and no *Pythium* was isolated from healthy plants (data not shown). Additionally, all samples were carefully screened for the presence of *Phytophthora* because of regional concerns; however, no collected plant or soil samples contained *Phytophthora*.

DISCUSSION

Within a stand of adult *B. noduliflora*, 85% of the individuals within our study site displayed signs of stress, dieback or death. The site had burned the year prior to the start of our study and most of the plants were resprouting and recovering following the fire. Additionally, most of the plants at this site had basal lignotubers that were quite large, suggesting that many of these plants were rather old and had persisted at the site for many years and had likely survived and successfully resprouted from previous fires. Resprouting plants that were similarly aged and burned in the same fire but were outside of the dieback area remained visibly healthy, suggesting that neither the fire nor a widespread event, such as drought, were responsible for the dieback. There was no difference in plant height, basal area, number of shoots or estimated burn intensity among the plants that remained healthy compared with co-occurring plants that were stressed or displayed crown dieback.

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Table 1. Number of isolates of fungal morphospecies and types obtained from healthy and stressed *Brunia noduliflora* plant tissues sampled from within and adjacent to a patch of *B. noduliflora* showing signs of stress, dieback and whole plant death in March 2009 ($n = 15$ for all organs from both stressed and healthy individuals)

	Number of isolates					
	Stressed individuals			Healthy individuals		
Fungal morphotypes	Root	Stem	Leaf	Root	Stem	Leaf
Pythium sp.						
Morphotype 1	15	–	–	–	–	–
Unidentified hyphomycetes						
Morphotype 1	1	–	2	–	–	–
Morphotype 2	2	1	–	–	–	–
Morphotype 3	–	3	–	3	1	–
Morphotype 4	–	1	1	–	–	–
Morphotype 5	2	1	–	1	–	–
Unidentified yeast						
Morphotype 1	–	–	–	3	–	–

The data suggest that differential fire intensity or fire recovery, and plant size/age were not chief drivers of plant mortality.

Water stress due to drought does not appear to be the primary factor leading to plant dieback and death among *B. noduliflora* at this site. It is not unusual that evergreen sclerophyllous shrubs in Mediterranean-type environments reach a permanent wilting point and lose turgor during the summer dry season (Pratt *et al.* 2007). However, this was not the case in the present study. At predawn, both healthy and stressed plants had water potentials well above the turgor loss point of *B. noduliflora*, suggesting that they had roots in contact with moist soil layers. Predawn water potentials were more akin to those found in riparian species in this region (Swift *et al.* 2008) rather than upland evergreen plants and water potentials were far less negative than previously reported values for this same species at a drier site (–4 MPa, Jacobsen *et al.* 2007). At midday, healthy plants maintained water potentials that were not different from their turgor loss point, whereas stressed plants were slightly below the turgor loss point. These results must be viewed with caution, however, because we did not measure turgor loss point of stressed plants and stress often triggers a lowering of the turgor loss point through osmotic adjustment or changes in tissue modulus of elasticity (Augé *et al.* 1986; Alarćon *et al.* 1993; Rodrigues *et al.* 1993). If such adjustments had taken place, it is possible that at midday the stressed plants may also have water potentials at or near their turgor loss point even though they had slightly lower midday water potentials than healthy plants. Additionally, the difference in water potential between predawn and midday was 1.01 ± 0.06 MPa for the healthy and 1.11 ± 0.11 MPa for the stressed plants. This value is directly proportional to transpiration rate and inversely proportional to plant leaf specific hydraulic conductance (k_l). Assuming that the healthy and stressed plants had similar transpiration rates, they would also have similar k_l values. In summary, it is unlikely that plants at the present study site were suffering significant strain because of drought-induced water stress. To the contrary, plants appeared to be well hydrated even though it was near the end of an extreme dry season.

It has been suggested that protracted drought could lead to carbon starvation of plants that close stomata during drought events in order to maintain isohydric water status (McDowell *et al.* 2008). Direct examination of the potential impact of carbon starvation is difficult and, to date, no study has been able to present conclusive evidence demonstrating carbon starvation as the direct cause of plant mortality (Sala *et al.* 2010); however, in the present study, the lack of a long-term drought in the region (2007, 2008 and 2009 all had precipitation totals at or above the long-term average for the site) and the high water status of plants even in the face of a short-term drought suggest that carbon starvation is an unlikely cause of plant stress. In the present study, even at the end of a drier than average dry season, both healthy and stressed plants were relatively hydrated and appeared to be active and transpiring during the day (as suggested by the change in water potential from predawn to midday). A decline in water potential of approximately 1 MPa from predawn to midday is the pattern typically seen in hydrated and active Mediterranean-type climate region evergreen shrubs, but not in isohydric water-stressed plants (Pratt *et al.* 2007). Additionally, both healthy and stressed

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plants had water potentials that were significantly higher than their turgor loss point at predawn. This suggests that stomata were likely open for some of the day in both groups, as stomatal closure has been shown to be correlated to the turgor loss point (Brodribb & Holbrook 2003; Brodribb *et al*. 2003). Moreover, leaves were examined microscopically from all experimental groups and no occlusions, fungal or otherwise, were observed that would prevent normal gas exchange. Combined, this makes carbon starvation an unlikely cause of the observed plant stress.

While water stress was likely not the proximate cause of plant stress, the ultimate cause of plant mortality appears to be runaway cavitation as plants became extremely dry and eventually died when they were no longer able to draw adequate moisture from the soil to replace water lost. This occurred when plants had already been heavily impacted and showed an advanced level of canopy dieback (>60% canopy dieback; Fig. 2) and likely had significant root loss as well. Thus, plant stress and initial canopy dieback appeared to be pathogen-induced in the present study. The lack of a difference in water potential in early stages of infection (i.e. between presumably non-infected healthy plants and infected stressed plants) varies from trends reported for plants infected with *Phytophthora cinnamomi* (Dawson & Weste 1989) but is consistent with results reported for other pathogenic species, in which plant performance is relatively little affected in early stages of infection even when there has been considerable root loss (Fleischmann *et al*. 2001).

A *Pythium* species was isolated from the roots of all sampled stressed plants in both 2009 and 2010 and was isolated from soil at the site. This species was not isolated from the roots of any sampled healthy plants. *Pythium* species are common soil fungi that are globally distributed and vary in their virulence. Plant species are also differentially susceptible to *Pythium* infection (Higginbotham *et al*. 2004; Augspurger & Wilkinson 2007). *Pythium* species have been known to cause root rot in a variety of species including tropical tree species (Augspurger & Wilkinson 2007), turfgrasses (Nelson & Craft 1991), olive trees (Hernández *et al*. 1998), temperate trees (Packer & Clay 2000) and crops, including wheat (Higginbotham *et al*. 2004), beans (Pieczarka & Abawi 1978) and spinach (Gold & Stanghellini 1985).

Pythium infection of *B. noduliflora* matches the pattern seen in plant stress, dieback and mortality observed at our study site. Infection and progressive loss of roots in stressed plants increases hydraulic resistance in stressed plants, perhaps because of root death or occlusion of xylem vessels, and this may be manifest as plants in the early stages of dieback also have lower midday water potentials compared with healthy plants. Eventually, this may also lead to lowered carbon stores and limited carbon gain, which would reduce plant ability to fight attack and would increase the rapidity of root loss. Progressive root death leads to decreased ability of plants to meet hydraulic needs, resulting in lowered water potentials as hydraulic supply becomes more limited and eventually leads to complete hydraulic failure.

The patchiness of the dieback and its spread to plants adjacent to the original patch from 2009 to 2010 are also indicative of a pathogenic cause of plant mortality. The area of dieback was contained to a patch of approximately 60 m by 180 m in 2009 and this patch size increased to approximately 64 m by 260 m in 2010. Drought-induced water stress would likely have affected a much broader area, provided that soil conditions are relatively constant regionally, and would not have expanded over the course of the following year and following an average rainy season.

We observed mortality in all of the resprouting shrub species found at our study site, including *B. noduliflora*, *L. salignum* and two *Erica* species. Other patches of mortality in stands of similar species have also been reported in the Western Cape. A large patch of dieback containing several 15–20 m circular areas of dieback of *Leucadendron rubrum* was reported previously at another location within the Paarl Mountain Nature Reserve (Milton 2003). Preliminarily, this was reported as being a potential site of pathogenic fungal infestation (Milton 2003), and may be an additional area affected by *Pythium*. Additionally, a previous case of mortality and dieback in *B. noduliflora* and *L. salignum* was reported in July 2008 at another site in the Western Cape (Rebelo *et al*. 2009). The pattern at this site was consistent with the pattern at our site, in that the plant death followed an above average rainfall winter. More rain than normal may facilitate reproduction and dispersal of *Pythium* propagules, which are most abundant in winter and in moist soil (Ali-Shtayeh *et al*. 1986; Martin & Loper 1999). A few dead plants were excavated at the site investigated by Rebelo *et al*. (2009) and they displayed abnormally few lateral roots. This was interpreted as potentially because of root death caused by asphyxiation and water logging, but is also consistent with *Pythium* root rot infection (Higginbotham *et al*. 2004).

Phytophthora cinnamomi (a genus closely related to *Pythium*) has been associated with death of native shrub species including members of the Proteaceae and Ericaceae (Von Broembsen & Kruger 1985); however, in the present study no *Phytophthora* was isolated (although we specifically assayed for it on three separate occasions from plant samples in 2009 and 2010 and from soil in 2010).

While our study did not explicitly test the pathogenecity of *Pythium* on the native shrub species observed to be stressed and dying, dieback of these

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species was strongly associated with infection by *Pythium*. This suggests that native fynbos shrub species may be susceptible to *Pythium* and this may represent an additional pathogenic threat to native plant communities in South Africa. Although *Pythium* species are globally distributed, death of these locally abundant and well-established plants suggests that this may be a fungal species introduced to the area that is not a locally native species. Within the initial dieback patch, stressed shrubs and shrubs with dieback declined from 2009 to 2010 and shrubs that were initially categorized as healthy in 2009 had begun to appear stressed by 2010. We observed no apparent recovery or reversal of shrub decline at the site.

The present study suggests that *Brunia* (Bruniaceae), *Leucadendron* (Proteaceae) and *Erica* (Ericaceae) may be susceptible to *Pythium* infection. These are genera within diverse, wide-spread and emblematic families of the fynbos ecosystem. Declines in the health, fitness and density of these dominant woody species are likely to have large impacts on the health of other species within affected communities and have the potential to affect ecosystem health. Additionally, other ecosystems that are dominated by closely related species, such as the flora of southwestern Australia, may also be particularly susceptible to this pathogen as well as the many communities and species worldwide that have already shown susceptibility to *Phytophthora*. Introduction of novel pathogens to natural communities may additionally threaten the incredibly biodiverse fynbos biome that is already extremely threatened by climate and land use changes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Monthly precipitation at the study site.

Appendix S2. Plants displaying differential stress and dieback.

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Supplemental Figure 1

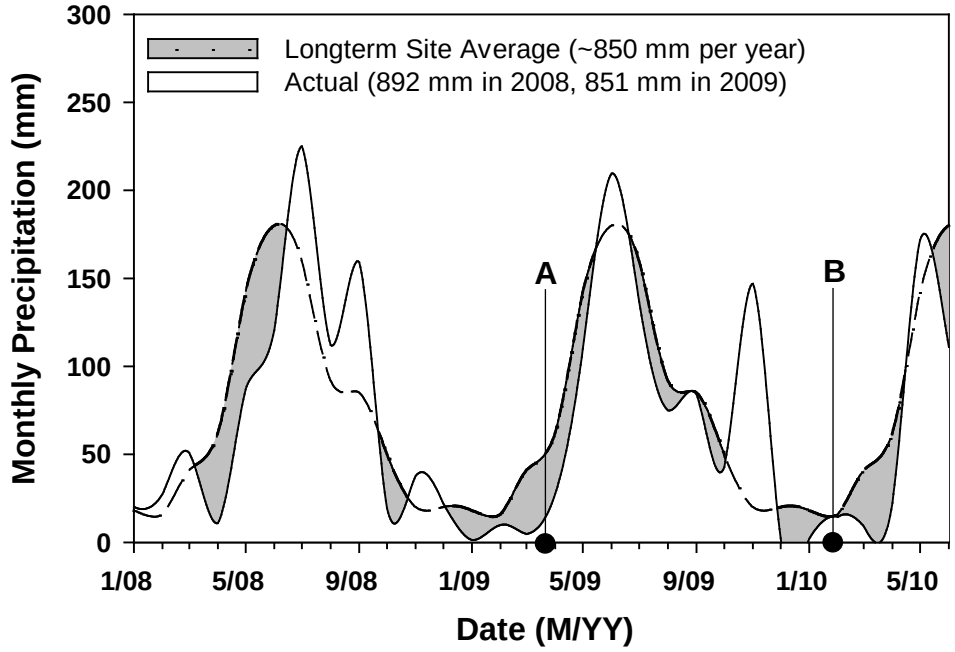

Fig. S1. Monthly precipitation for the study site located within the Paarl Mountain Nature Reserve, Western Cape, South Africa including the long-term site average precipitation (grey fill and dashed line; data from Milton 2003) and the actual monthly precipitation of the field site from January 2008-June 2010 (white fill and solid line). Actual values for the site were calculated as the mean of precipitation data collected from two nearby weather stations (Bellevue and Fairview) operated by the Agriculture Research Council of South Africa. Dieback of *Brunia noduliflora* was originally observed and sampled at time "A," following a period of lower than normal summer rainfall. Sampling the following year was conducted at time "B."

Supplemental Figure 2

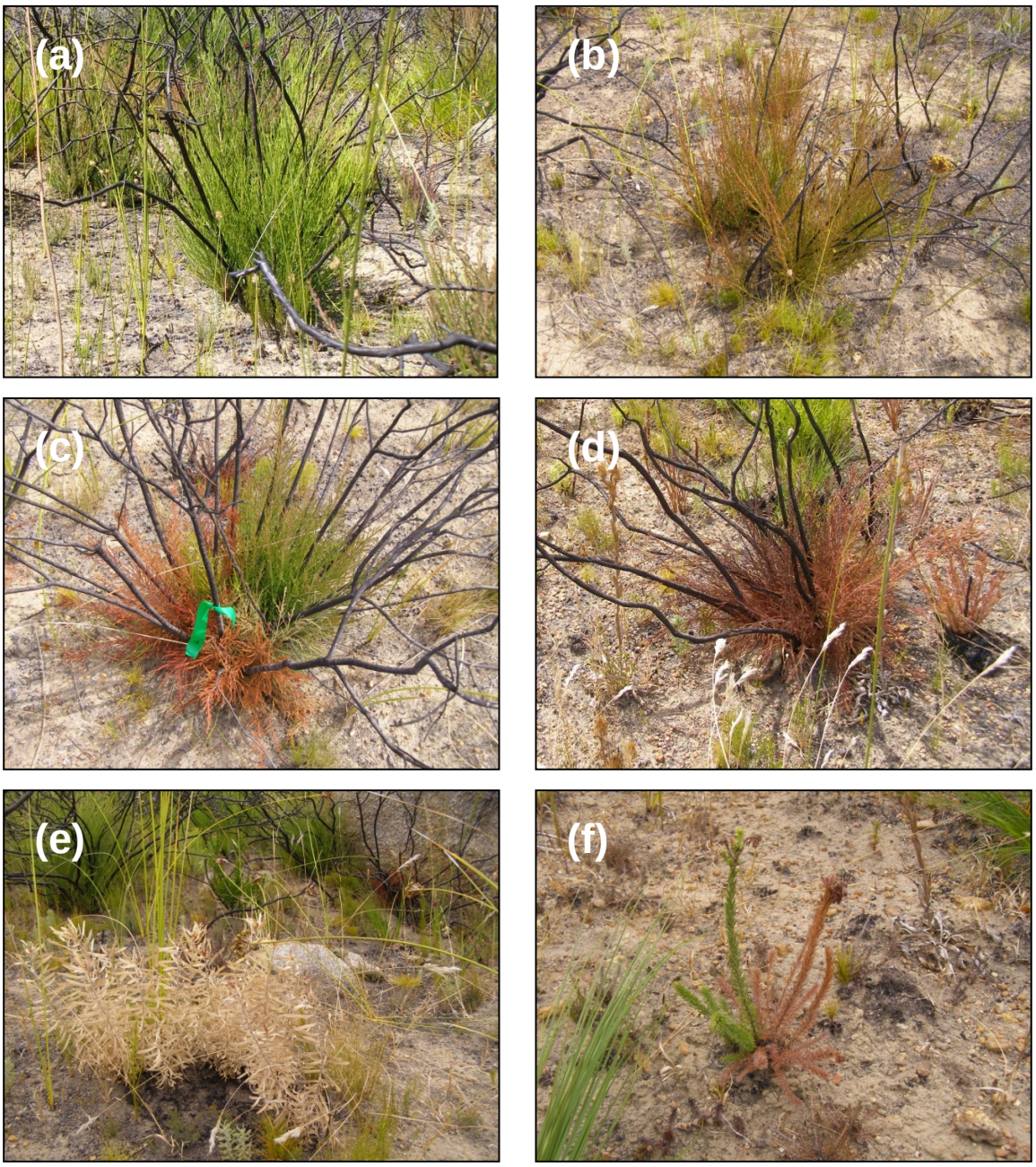

Fig. S2. *Brunia noduliflora* plants from a site located within the Paarl Mountain Nature Reserve, South Africa displaying differential stress and dieback. Individuals of *B. noduliflora* within the site ranged from healthy (a), to showing signs of stress with reddening and yellowing leaves (b), to partial canopy dieback (c), and complete

22 crown death (d). Other woody shrub species within the site also showed canopy death
23 and dieback including *Leucadendron salignum* (e) and an *Erica* sp (f).
24