# The life cycle and field epidemiology of *Uromycladium acaciae* (Pucciniales) on *Acacia mearnsii* in South Africa

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## Key Words

Spore traps, disease development, spore dispersal, water-splash, niche differentiation, fungi

## Abstract

*Uromycladium acaciae* has damaged plantations of *Acacia mearnsii* in southern Africa since 2013. Uredinia of a species of *Uromycladium* have been known on *A. mearnsii* in South Africa since the 1980s. However, the recent damage is associated with telia and spermogonia. Uredinia and telia were previously treated as conspecific with a phylogenetic species concept. However, uredinia did not form after previous artificial inoculation experiments with teliospores. Controlled studies identified the optimum conditions for basidiospore infection, but the optimum conditions for sporulation and dispersal have not been identified. To investigate the life cycle and field epidemiology of *Uromycladium* on *A.*

*mearnsii*, spores were trapped weekly and development of disease symptoms and plant phenology were monitored monthly at three plantations. Telia and spermogonia developed independently from uredinia, and nucleotide polymorphisms between rDNA of uredinia and telia were fixed based on high throughput sequencing and PCRs. All three weather variables measured had a significant effect on teliospore abundance at two of the three sites. Teliospore abundance was greatest during trapping periods when mean relative humidity was high, mean rainfall was 4-5 mm $day^{-1}$, and mean temperature was 15-16°C. Teliospore counts peaked at the end of summer, potentially the result of epidemic build-up. Results support the hypothesis that despite sharing a most recent common ancestor, uredinia on *A. mearnsii* in southern Africa are independent to the life cycle of the telial rust, which likely constitutes a new introduction. Furthermore, teliospores of *U. acaciae* disperse under wet conditions; and, the wet season between October and March is the optimal period for wattle rust development.

## Introduction

Wattle rust, caused by *Uromycladium acaciae*, is the most economically important disease currently affecting plantations of *Acacia mearnsii* (black wattle) in southern Africa (McTaggart *et al.* 2015; Little & Payn 2016; Fraser *et al.* 2017). The disease was first observed in the KwaZulu-Natal Midlands in 2013 and has been reported from plantations throughout KwaZulu-Natal, Mpumalanga and Eswatini (formerly Swaziland), as well as from jungle stands in Limpopo, the Eastern Cape, and eastern regions of the Western Cape (McTaggart *et al.* 2015, Fraser *et al.* 2017). Symptoms and signs of the disease include rachis and rachilla (leaf midrib) malformation (or folding), production of chocolate-brown telia that become “slimy” in wet weather, matting of leaves, and stunting of growth

(McTaggart *et al.* 2015; Fraser *et al.* 2017). Little & Payn (2016) estimated growth losses to young trees of 20–40% caused by *U. acaciae*.

*Uromycladium acaciae* was first described as *Uredo acaciae* from telia and teliospores on an Australian species of *Acacia* in New Zealand (Cooke 1890). It was later combined to *Uromycladium* and treated as conspecific with *Uromycladium bisporum* (Sydow & Sydow 1915). *Uromycladium bisporum* was described from telia and teliospores collected on *Acacia dealbata* in Australia (McAlpine 1905). Uredinia and spermogonia were not described for *U. acaciae* or *U. bisporum*. Gadgil & Dick (1999), Dick (2009) and Berndt (2010) also referred to *U. acaciae* as a microcyclic rust. However, McTaggart *et al.* (2015) epitypified ribosomal DNA sequences of *U. acaciae* from a specimen with spermogonia and telia and described uredinia associated with telia of *U. acaciae* in South Africa.

Uredinia of a species of *Uromycladium*, initially identified as *U. alpinum*, were first observed on *A. mearnsii* in South Africa in the 1980s (Morris *et al.* 1988). McTaggart *et al.* (2015) treated this rust as the uredinial stage of *U. acaciae*, based on a phylogenetic species concept using the ITS (internal transcribed spacer) and LSU (large subunit) regions of ribosomal DNA (rDNA). Single nucleotide polymorphisms (SNPs) and indels in these rDNA regions were attributed to intraspecific differences between uredinial and telial specimens. McTaggart *et al.* (2015) noted that only uredinia were found in the Western Cape and Limpopo Provinces at the time of their survey. In their study, sequences of uredinia came from the Western Cape, while telia came from KwaZulu-Natal. As yet, no uredinia have been sequenced from areas where telia are also present. Subsequently, Fraser *et al.* (2017) observed that only spermogonia and telia developed after artificial inoculations with teliospore suspensions. They hypothesised that the uredinial stage may not be part of the

life cycle of *U. acaciae*, and that two closely related taxa in *Uromycladium,* one with a uredinial state and the other with a telial state, and that share a most recent common ancestor, occur on *A. mearnsii* in southern Africa.

Fraser *et al.* (2017) found that teliospores of *U. acaciae* germinated in water to produce basidia with four basidiospores. Basidiospore production was not observed within telia, even under damp conditions (Fraser *et al.* unpublished data). Teliospores are therefore thought to act as diaspores (dispersal units) as has been proposed for other species of *Uromycladium* (Morris 1997; Dick *et al.* 2009; Rahayu *et al.* 2010). Young telia of *U. acaciae* are pulverulent (powdery) but become slimy under moist conditions, as hygroscopic cysts at the base of teliospores swell and burst (Fraser *et al.* 2017). These observations suggest that teliospores of *U. acaciae* are likely to disperse locally in water-splash or further within micro-droplets liberated and spread during windy rain periods (Fraser *et al.* 2017). This is in comparison to dry dispersal in wind, which has been suggested for other species of *Uromycladium* (Morris 1997; Rahayu *et al.* 2010).

Although Fraser *et al.* (2017) identified the optimal environmental conditions for teliospore germination and basidiospore infection of *A. mearnsii* by *U. acaciae*, nothing is known about the impact of environmental conditions on other key processes, such as latent period, sporulation, or dispersal. Fraser *et al.* (2017) identified the optimum conditions for basidiospore infection as more than 12 hours of leaf wetness at 15–20°C. They postulated that climatic conditions during the wet season in the KwaZulu-Natal and Mpumalanga Provinces, between October and March, would be ideal for epidemics of *U. acaciae*. Furthermore, the wet season also favours growth of *A. mearnsii*, and only young host tissue is susceptible to infection by *U. acaciae* (Fraser *et al.* 2017).

The life cycle and field epidemiology of *U. acaciae* have not been fully elucidated. This hinders the development of an informed strategy for disease control. Understanding the life cycle and epidemiology of *U. acaciae* will improve the efficacy of chemical control, resistance screening through artificial inoculation (Fraser *et al.* 2019), and disease risk modelling and forecasting.

In this study, spores of *U. acaciae* were trapped and development of disease symptoms and plant phenology monitored at three plantations of *A. mearnsii* in the Mpumalanga Province of South Africa to test three hypotheses relating to the above knowledge gaps: (i) uredinia and telia of *Uromycladium* on *A. mearnsii* in southern Africa occur in separate life cycles, they will therefore develop independently in the field, and nucelotide polymorphisms will be fixed between ribosomal DNA of telia and uredinia; (ii) teliospores of *U. acaciae* act as diaspores (rather than basidiospores) and are mainly dispersed under wet conditions, therefore high numbers will be trapped during periods of high relative humidity or rainfall; and, (iii) the wet season between October and March will be the most suitable period for wattle rust development.

## Materials and methods

### Field sites

Three 1-3 year old plantations (Dundonald, Iswepe, and Moolman) of *A. mearnsii* in mist belt areas of the Mpumalanga Province were selected for spore trapping, as well as monitoring of disease symptom development and host phenology (Table 1). *Uromycladium acaciae* was present at all sites at trial establishment. Three spore traps were placed at each site in January or February 2016. Traps were placed between, and within close

proximity of two visibly infected trees within a row. Three trees at each site, one alongside each spore trap, were selected for disease and phenology monitoring. Spore traps were placed at least 20 m apart and at least 20 m away from the plantation edge. Temperature and humidity loggers (Maxim iButtons DS1923, Fairbridge Technologies, Johannesburg) were attached to the inner crown of each monitored tree. Temperature and relative humidity were recorded hourly. Precipitation was measured weekly with one rain gauge at each site from 26th August 2016 onwards. Precipitation data for trapping periods prior to this date were acquired from farms within 10 km of the Dundonald and Moolman sites. Precipitation data for the farm adjacent to the Moolman site was also used for the Iswepe site (c. 40 km away), as no closer reliable weather data were available.

**Spore trapping**

Spore traps were similar in design to those described by Schoeman *et al.* (1995). Each spore trap consisted of four petroleum jelly (Vaseline) coated microscope slides affixed to a wooden frame, consisting of two strips of wood fixed at right angles to one another on top of a 1 m wooden pole (Fig. 1). Slides were coated with petroleum jelly on one side, following the method of Ostry & Nicholls (1982). Slides were attached horizontally (Vaseline coated side facing upwards) and were held in place at the end of the wooden strips with clothes pegs. Slides were changed weekly, and spore numbers assessed under a light microscope (Axioskop, Zeiss). The total number of teliospores and urediniospores were recorded in five vertical transects (along the width of the slide) at a magnification of x10. To avoid pseudoreplication, the total counts of teliospores and urediniospores per slide were each averaged to give a mean value for each trap over a trapping period. To

account for some variation in trapping period lengths, the numbers of spores per day of trapping were used in statistical analysis.

**Monitoring of plant phenology and development of symptoms and signs**

Plant phenology and development of rust symptoms and signs were monitored on three trees at each site from 19th April 2016 until 7th April 2017. Three trees at each site, one beside each spore trap, were selected and six branches at breast hight on each tree were labelled. Each month the branch tip was marked with tape and the growth and visible rust development from the previous month assessed. The following factors were recorded: number of fully expanded leaves (leaves refers to whole compound leaves made up of numerous leaflets and pinnules), number of emerging leaves, and the presence or absence of telia, spermogonia and uredinia on both leaf growth stages. Samples of up to ten leaves with symptoms from the most recent growth, either fully expanded or still expanding depending on time of year, were collected monthly from random branches on each tree and inspected under a dissection microscope (Discovery.V12, Zeiss). Different life stages were identified morphologically under the microscope and the presence or absence of spermogonia, telia and uredinia on each leaf was recorded. The proportion of leaves with different combinations of rust life stages was recorded. For example, whether telia, telia and spermogonia, or telia, spermogonia and uredinia were present on a leaf.

**DNA extraction and sequencing**

To test the prediction that polymorphisms in rDNA are fixed between uredinia and telia, sequences of the LSU region of uredinia collected from two of the monitoring sites were compared to telial and uredinial sequences published by McTaggart *et al.* (2015). DNA was

extracted from single uredinia collected from both the Dundonald and Moolman sites in July 2016 with the UltraClean Microbial DNA Isolation Kit (MoBio Laboratories Inc., Solana Beach, CA, USA). The LSU region of rDNA, which contained one SNP between uredinia and telia observed by McTaggart *et al.* (2015), was amplified from uredinia. PCRs were amplified with FastStart Taq (Roche Diagnostics Corporation, Indianapolis, USA) following the manufacturer's instructions. The PCRs were amplified with the primers Rust2INV (Aime 2006) and LR7 (Vilgalys *et al.* 1990) at an annealing temperature of 62°C. PCR products were cleaned by an ethanol precipitation and sequenced in both directions using an ABI PRISM Dye-Terminator Cycle Sequencing Kit (Applied Biosystems) on an automated ABI 3130xl sequencer at the DNA Sequencing Facility of the Faculty of Natural and Agricultural Sciences, University of Pretoria. Sequences were assembled using the CLC Main Workbench (Qiagen).

We tested for within-genome variation in rDNA with high-throughput sequencing from a telial isolate of *U. acaciae*, specifically to examine whether polymorphisms from uredinia were present in copies of rDNA from telia. We expected to observe diversity within copies of the ITS region from one isolate as teliospores have two independent nuclei (dikaryotic), both of which have multiple repeats of rDNA (Virtudazo *et al.* 2001; Feau *et al.* 2011; Persoons *et al.* 2014; McTaggart and Aime 2018; Rush *et al.* 2019). Parts of rDNA are used by the mycological community as barcode loci and intraspecific diversity is expected to be less than interspecific diversity. DNA was extracted from a single-pustule isolate of a telium collected from Hilton, Pietermaritzburg, KwaZulu-Natal in 2015 (Fraser *et al.* 2017) using the Machery Nagel NucleoSpin Plant II Kit. Multiple extractions were pooled with less than 5mg of tissue per extraction to account for the high viscosity of the telial suspension. The

resulting DNA was concentrated and further purified through an additional silica spin column. Approximately 1 µg of DNA was submitted to the DNA Sequencing Facility of the Faculty of Natural and Agricultural Sciences, University of Pretoria, for library preparation and IonTorrent sequencing on a 530v1 chip. We used MITObim v1.9.1 (Hahn *et al.* 2013) to assemble rDNA from the raw data with a starting seed of the LSU region (KR612242), and extracted reads that mapped to this contig with BBSplit in the BBMap package (available at: https://sourceforge.net/projects/bbmap/). These reads were mapped to the assembled rDNA in Geneious Prime (https://www.geneious.com) using the Geneious mapper tool (Supplementary Files). We searched the mapped reads for polymorphisms at five SNP and indel sites identified in a previous study (McTaggart *et al.* 2015).

**Statistical analysis**

Statistical analysis was performed in R version 3.6.1 (R Development Core Team 2019). To visualise the relationship between season and spore abundance, counts of the two spore types were plotted against time. Weather variables and host phenology were also plotted against time. Similarly, the proportion of samples with different combinations of rust life stages co-occurring was plotted against time. Trend lines were added to all plots with locally weighted smoothing (LOESS).

To analyse the relationship between weather variables and teliospore and urediniospore abundances, individual Poisson generalised additive models (GAMs) (negative binomial with a log link was applied because of overdispersion) were constructed separately for each spore type. Investigated weather variables were averages within trapping period lengths as follows: average temperature (°C); average relative humidity (%) and average daily rainfall (mm). The negative binomial GAM comprised site as a parametric model

component and a smooth term for the weather variables for each site. The models were as follows:

$$\log\left(\mathrm{E}(\mathrm{y}_{ikl})\right)_j = \alpha_{(j)} + f_{(j)}(\mathrm{temperature}_{ik}):\mathrm{site}_{(j)ikl} + f_{(j)}(\mathrm{humidity}_{ik}):\mathrm{site}_{(j)ikl}$$

$$+f_{(j)}(\mathrm{rainfall}_{ik}):\mathrm{site}_{(j)ikl}, \quad (1)$$

where $\log(\cdot)$ is the log link function; $\mathrm{E}(\mathrm{y}_{ikl})$ is the expected count at trap $i$ ($i = 1,2,3$) and trapping period $k$ ($k = 1,\ldots,61$); $l = 1,2,3$ indicates the category of the variable site; $j = 1,2$ indicates the $j$th additive predictor for teliospore and urediniospore respectively; $\alpha$ is an intercept and $f(\cdot)$ is a smooth function.

The negative binomial GAM models in equation (1) were fitted by penalized likelihood, with cubic regression splines being used as smooth terms using the mgcv package (Wood & Wood 2015). For urediniospore data, outliers and extreme observations in the counts (response) were detected by plots of residuals versus fitted values and plots of response variable against fitted values, raw data of suspect observations in the counts were then checked and if confirmed as an outlier/extreme observation they were subsequently dropped from further analysis (Wood 2017). Graphical validation of the negative binomial model in equation (1), such as plots of Pearson residuals against the fitted values and versus each explanatory variable in the model and plots of ordered deviance residuals against their theoretical quantiles, indicated that the assumed mean-variance relationship was reasonable and there were no patterns in the residuals. An auto-correlation for the (normalised) residuals for each individual time series (for each time series in each trap within site) calculated from the negative binomial model in equation (1) indicated that there was no temporal correlation within each of residual time series. Model comparisons

were based on the Akaike Information Criterion (AIC). Maximum likelihood (ML) smoothing parameter estimation discussed in Wood (2017) was used for model selection. Concurvity used to identify which smooths can be approximated by any combination of the other smooths in the model was evaluated using the mgcv package. Concurvity is analogous to collinearity in generalised linear models (GLMs) and causes similar problems of statistical inference. The pairwise concurvities obtained after GAM exact estimations indicated that the degree to which each variable was approximated by each other variable did not exceed ~0.26.

## Results

### Disease development and rust life stages

Spermogonia, telia and uredinia were observed on samples collected from all three sites. Spermogonia and telia were the dominant life stages observed and were recorded at all time points. Uredinia were also observed at most time points, but on fewer samples. Spermogonia and uredinia were never observed co-occuring on the same leaf without telia also being present, and uredinia were never observed in close association with spermogonia. There was some seasonal variation in the proportion of samples of the freshest growth with different combinations of the three life stages (Fig. 2). Spermogonia and telia were found to co-occur without uredinia on most samples collected during the wet (growing) season, between October and March. The proportion of samples on which uredinia co-occurred with telia increased during the dry season (winter). This finding was similar to the observation that spermogonia and telia developed on young plant tissues, such as expanding leaves, but that uredinia developed only on older plant tissues, such as

fully expanded leaves (Fig. 1). Plant tissues collected in winter were older than those collected in summer, given that the plants were not actively growing in this period.

All three life stages were observed on both sides of leaves, however telia were observed mostly on abaxial leaf surfaces, while spermogonia and uredinia were mostly observed on adaxial leaf surfaces. Uredinia were dark brown to black in colour and were noticeably darker than the chocolate-brown telia. Uredinia were often, but not always, observed within yellow lesions (Fig. 1). This was not the case for spermogonia or telia. Both uredinia and telia were produced dry, however, under wet conditions, teliospores exuded in slimy masses and coated the plant tissues (Fig. 1).

**Seasonal patterns of spore abundance on traps**

Teliospores and urediniospores were observed on spore traps at all trapping periods across all three sites (Fig. 3). Generally, counts of teliospores (mean 37.5 ± 3.2 spores $day^{-1}$) were far greater than of urediniospores (mean 4.4 ± 0.4 spores $day^{-1}$). Counts of both spore types varied among sites, among traps at the same sites, and among trapping periods.

Counts of teliospores were greatest at the Dundonald (mean 51.7 ± 7.0 spores $day^{-1}$) and Moolman (mean 43.8 ± 6.0 spores $day^{-1}$) sites, and lowest at the Iswepe site (mean 17.6 ± 1.7 spores $day^{-1}$). There was some variation in the pattern of teliospore counts among the three sites, but generally it was similar (Fig. 3). At all three sites, the greatest abundance of teliospores was observed towards the end of the wet season in February-March 2017. Other smaller peaks were observed in April 2016 at Dundonald and Moolman, June-July 2016 at Moolman, October 2016 at Dundonald and January 2017 at Dundonald and Moolman.

Counts of urediniospores were greatest at the Dundonald site (mean 9.2 ± 1.1 spores day$^{-1}$) and lower at the Moolman (mean 2.0 ± 0.2 spores day$^{-1}$) and Iswepe sites (mean 2.3 ± 0.2 spores day$^{-1}$). At Moolman and Iswepe, counts of urediniospores remained low throughout the trapping period. In contrast, several peaks in the abundance of urediniospores were observed at the Dundonald site. The greatest peak was observed in March 2016, with smaller peaks in April 2016, July-September 2016, and January-April 2017 (Fig. 3).

**Seasonal patterns of host phenology**

The number of newly emerging leaves per branch was greatest during the wet season (October to April) and lowest during the dry season (May to September) at all sites (Fig. 3). Number of emerging leaves peaked in January at the Dundonald and Iswepe sites, but slightly earlier in November at the Moolman site. Number of emerging leaves was lowest in June and July 2016 for all sites. Growth initiation was observed in August 2016 at all sites.

**Impact of weather variables on teliospore abundance on spore traps**

All three weather variables had a highly significant ($P < 0.001$; Table 2) or marginally significant ($P < 0.06$; Table 2) effect on teliospore abundance at the Dundonald and Moolman sites, but no weather variables were found to have a significant effect on teliospore abundance at Iswepe ($P > 0.05$; Table 2). Average temperature during the trapping period had a highly significant effect on teliospore abundance at the Moolman site ($P < 0.001$; Table 2) and a marginally significant effect on teliospore abundance at the Dundonald site ($P = 0.052$; Table 2). Average relative humidity and average daily rainfall during the trapping period both had a highly significant effect on teliospore abundance at

both the Dundonald and Moolman sites (all $P < 0.001$; Table 2). The model had an explained deviance of 31.3% (i.e. weather variables and site explained 31.3% of the variation in teliospore abundance).

Teliospore abundance at Dundonald increased with increasing average temperature up to about 15-16°C and then started to decrease (Fig. 4a). Whereas, the estimated smooth for the Moolman site exhibited three significant peaks in teliospore abundance; the first at an average temperature of 11°C, the second at an average temperature of about 16°C and the third at the temperature of about 18°C (Fig. 4c).

At Dundonald, teliospore abundance increased non-linearly with increasing average relative humidity (Fig. 4d). At Moolman, the estimated smooth started to increase above a relative humidity of 80% (Fig. 4f).

At both Dundonald and Moolman, the estimated trends for teliospore abundance against average daily rainfall were very similar, with abundance decreasing steeply between rainfall of 0 and 2 mm $day^{-1}$, and starting to increase between the rainfall of 2 and 4 mm $day^{-1}$, and reaching a peak abundance at the rainfall of 4-5 mm $day^{-1}$ and then starting to drop (Fig. 4g, 4i).

**Impact of weather variables on urediniospore abundance on spore traps**

All three weather variables tested had a highly significant effect on the abundance of urediniospores at the Dundonald site (all $P < 0.001$; Table 3) and a significant effect at the Moolman sites (all $P < 0.01$; Table 3), but no weather variables were found to have a significant effect on urediniospore abundance at Iswepe ($P > 0.05$; Table 3). The model had

an explained deviance of 45.2% (i.e. weather variables and site explained 45.2% of the variation in urediniospore abundance).

The estimated smooth for Dundonald exhibited two significant peaks in urediniospore abundance against average temperature; a first peak at an average temperature of 11-12°C, and a larger one at about 17-19°C (Fig. 5(a)). At Moolman, urediniospore abundance increased in a non-linear pattern below and above an average temperature of about 15°C (Fig. 5(c)).

At Dundonald, estimated urediniospore abundance increased with increasing relative humidity, reached a peak abundance at an average relatively humidity of 68%, then decreased slightly and stayed fairly stable (Fig. 5(d)). At Moolman, urediniospore abundance peaked at a relative humidity of about 60% and then again above 90% (Fig. 5(f)).

At Dundonald, estimated urediniospore abundance decreased steeply between average daily rainfall of 0 and 2 mm $day^{-1}$, and started to increase between 2 and 4 mm $day^{-1}$, and reached a peak abundance at about 4-5mm $day^{-1}$ (Fig. 5(g)). At Moolman, urdeiniospore abundance increased with increasing average daily rainfall until about 8-9 mm $day^{-1}$ before decreasing (Fig. 5(i)).

**DNA sequence analysis**

The LSU sequences obtained from uredinia of *U. acaciae* from Mpumalanga had 100% identity to the uredinia from the Western Cape sequenced by McTaggart *et al.* (2015). These differed from telial sequences from KwaZulu-Natal by one SNP in the LSU region (Fig. 6).

Approximately 19 million reads with a mean length of 271 bp were obtained from IonTorrent sequencing of the single telium isolate. The rDNA region of *U. acaciae* was assembled as a 6,603 bp contig with a mean coverage of 2,615 mapped sequences (GenBank MT226400). There were six indel and SNP sites in reads that mapped to the ITS region, which showed there is intragenomic and intraspecific variation in rDNA of a single pustule isolate (Supplementary files). However, none of these SNPs or indels matched those observed in sequences from uredinia.

## Discussion

The two core findings of this study are that teliospores of *U. acaciae* mostly disperse under wet conditions and the wet season is most suitable for disease epidemics caused by *U. acaciae*. The results also support a hypothesis that uredinia do not form part of the life cycle of the telial rust on *A. mearnsii* in southern Africa. This knowledge will contribute to the development of strategies for disease control.

The hypothesis that uredinia and telia of *Uromycladium* on *A. mearnsii* are not part of the same lifecyle was supported based on field observations. Uredinia and telia developed independently at all three monitored plantations of *A. mearnsii* in Mpumalanga. Spermogonia and telia developed on young plant tissue, whereas uredinia were only observed on older plant material. Spermogonia and uredinia were never observed to co-occur on leaves without telia, whereas telia and spermogonia co-occurred frequently, with and without uredinia. Further, Spermogonia and uredinia were never observed in close association, however spermogonia and telia were usually bserved in close association. These observations are similar to those reported from previous controlled experiments, in

which only spermogonia and telia developed on seedlings of *A. mearnsii* after artificial inoculation with suspensions of teliospores (Fraser *et al.* 2017). Based on ontogeny of other rust fungi, uredinia would be expected to develop after dikaryotisation of spermogonia and before the production of telia If they were part of the same life cycle.

Support for this hypothesis also comes from rDNA sequence analysis. LSU sequences of uredinia from Mpumalanga that co-occurred with telia matched sequences of uredinia from the Western Cape and differed from sequences of telia from KwaZulu-Natal by one fixed SNP (McTaggart et al. 2015). While, a contig of rDNA assembled from high-throughput sequencing data of an isolate derived from a single telium (mean coverage >2,000 bp) showed intragenomic variation, but did not contain polymorphisms that were found in uredinia (two indels and one SNP in the ITS region, and one SNP in the LSU region), showing there was no recent admixture. McTaggart *et al.* (2015) attributed SNPs and indels in the ITS and LSU regions of rDNA to intraspecific differences, and treated uredinia and telia as conspecific based on a phylogenetic species concept. We have provided further evidence that these genetic differences are fixed between uredinia and telia. The telial stage of *U. acaciae*, whether a different lineage or species, likely represents a recent introduction to southern Africa, rather than a newly expressed life stage of the uredinial *Uromycladium*, identified as *U. alpinum* by Morris *et al.* (1988).

McTaggart *et al.* (2015) showed that the species of *Uromycladium* on *A. decurrens*, *A. mearnsii*, and *A. terminalis* in Australia and South Africa shared a most recent common ancestor. They consequently applied the name *U. acaciae* to the rust based on morphology of the type description. *Uromycladium acaciae* was epitypified by rDNA from a specimen with telia on *A. mearnsii* in KwaZulu-Natal, South Africa (McTaggart *et al.* 2015). Whether

*U. acaciae* is a complex of species remains a knowledge gap. However, intraspecific variability of rDNA is known in other species of *Uromycladium* (Doungsa-ard *et al.* 2018) and other rust fungi (Rush *et al.* 2019). Resolution of this taxonomic uncertainty and the possible existence of a complex of species will hinge on wide sampling of this rust in its native range and application of molecular markers other than rDNA.

Teliospores of *U. acaciae* were found on spore traps in high numbers, supporting the hypothesis that they act as diaspores (dispersal units) in common with other species of *Uromycladium*, rather than basidiospores. Fraser *et al.* (2017) proposed that hygroscopic cysts at the base of pedicels of teliospores of *U. acaciae* swell and burst when wetted to produce slimy spore masses and that teliospores may thus disperse during rainfall. This is supported by the positive relationship between relative humidity and teliospore counts reported here for two of the monitoring sites. However, the observed relationship between rainfall and teliospore counts was more complicated. Teliospore abundance peaked at 5 mm $day^{-1}$ rain, decreasing at higher values. This relationship may have been caused by wash-off from trap slides during heavy downpours. Teliospore abundance was also relatively high during several trapping periods without rain. This may be explained by the situation of the three monitoring sites in mist belt areas of Mpumalanga, with maximum relative humidity reaching above 98% during all but one trapping period. These misty conditions, with nightly dew formation, may explain why teliospores were detected during periods without rain. During periods of mist or fog, teliospores of *U. acaciae* will exude from telia and may be dispersed locally in water that accumulates on leaves and forms large droplets (Gregory *et al.* 1959).

To the best of our knowledge, water dispersal has been reported only for teliospores of one rust fungus, *Chrysomyxa weirii*, which causes spruce needle rust (Crane *et al.* 2000). There are some similarities in the behaviour of *U. acaciae* and *C. weirii*. Under dry conditions, *C. weirii* forms crusts of teliospores on needle surfaces around the sori; these masses dissolve and disperse when water is present (Crane *et al.* 2000). A mucilaginous, adhesive substance prevents the dispersal of teliospores by wind and protects spores from desiccation in dry weather (Crane *et al.* 2000). Similar crusts have been observed around telia of *U. acaciae* under dry conditions, following wet periods. A surface layer of teliospores forms a crust that appears to protect the teliospores below from desiccation until the next period of wet weather. Adaptations such as these mean that spores would be released only under conditions suitable for germination (Gregory *et al.* 1959).

Splash-dispersed fungal spores are wettable and usually have a smooth surface, thin hyaline walls and elongated shape (Fitt *et al.* 1989). Teliospores of *U. acaciae* are wettable and have a smooth surface but are pigmented and rounded. The proposed water splash dispersal mechanism of teliospores of *U. acaciae* contrasts with the dry dispersal of teliospores of *U. tepperianum* and *U. falcatarium* in wind (Morris 1997; Rahayu *et al.* 2010). Teliospores of these species have ornamented surfaces and no cysts on their pedicels. In contrast uredinia of *Uromycladium* on *A. mearnsii* are likely wind dispersed, having several of the characteristics of wind-borne fungi outlined by Fitt *et al.* (1989). They are borne dry, are non-wettable (Fraser, unpublished data), have an ornamented surface and are rounded. Nonetheless, there was a similar relationship between rainfall and urediniospore counts, as seen for teliospores. This may have been due to the liberation of urediniospores

by the impact of rain drops through the puff or tap mechanism (Hirst & Stedman 1963) or the positive covariation of temperature and rainfall.

Teliospores of *U. acaciae* were detected throughout the monitoring period, but the greatest numbers were observed between January and March 2017. This period corresponded with the second half of the growing season in Mpumalanga, where the climate is characterised by wet summers and dry winters. This observation supports the prediction of Fraser *et al.* (2017) that October to March would be ideal months for infection by *U. acaciae* in the regions of South Africa where *A. mearnsii* is cultivated. They reported that the optimum conditions for infection were 12 hours or more of leaf wetness at 15-20°C, and that only young expanding plant tissues were susceptible. Between October and March at the three monitoring sites, average temperatures were around 15-20°C, there was abundant rain and plentiful emerging leaves (Fig. 3). This period is clearly optimal for the wattle rust. The peak in teliospore abundance in the second half of this period could be the result of an epidemic build up in the first half of the growing season. Under controlled conditions, spermogonia and telia developed 2-5 weeks after inoculation (Fraser *et al.* 2017), suggesting that the peak in teliospore abundance may have been the result from 1-3 rounds of infections. One limitation of this study was that monitoring was undertaken for just over one year. Longer term monitoring would be required to investigate between-year variation in patterns of symptom development and sporulation.

Although not the focus of this study, some differences in spore abundance were observed among the different sites. Both teliospore and urediniospore abundance were greatest at Dundonald, while teliospore abundance was also greater at Moolman than at Iswepe. The low levels of both teliospores and urediniospores at Iswepe probably explains why the

relationships seen with weather variables at the other sites were not also seen here. There were several differences between the sites, thus determining the cause of among-site variation would be speculative. However, some differences can be highlighted based on the relationships between environment and pathogen reported here. Relative humidity and rainfall were lower at Iswepe than the other sites at the start of the wet season, while relative humidity and rainfall were greater, and temperatures lower, at Dundonald during the wet season. It must also be noted that Dundonald was an older plantation with a closed canopy. Importantly, seasonal patterns and relationships with weather variables were mostly consistent among sites.

Results of this study have added to the knowledge of the biology of *U. acaciae*, a little known, but important pathogen of *A. mearnsii* in southern Africa. A better understanding of spore dispersal processes and timing, as well as the lifecycle of the pathogen, will support the development of an integrated pest management programme, incorporating chemical and biological control tools, resistance breeding, and disease risk modelling and forecasting. The finding that telia and uredinia are part of separate life cycles on *A. mearnsii* in southern Africa shows that further research is required to resolve the identity of these taxa.

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## Tables

**Table 1** Details of plantations of *Acacia mearnsii* used for spore trapping and disease monitoring of *Uromycladium acaciae*

| Site | Dundonald | Iswepe | Moolman |
|---|---|---|---|
| Location | -26° 11' 20.4843", 30° 47' 24.6483" | -26° 56' 45.9175", 30° 31' 8.9370" | - 27° 7' 56.6260", 30° 55' 51.0928" |
| Land owner | KLF (SAFCOL) | Bruno Paul | TWK |
| Elevation (m) | 1698 | 1461 | 1264 |
| Plantation size | 25.9 ha | 14.1 ha | 9.1 ha |
| Soil | Clovelly sandy loam | Clovelly sandy loam | Avalon sandy loam |
| Normal mean annual increment of site | 8 t $ha^{-1}$ $yr^{-1}$ | 10 t $ha^{-1}$ $yr^{-1}$ | 12 t $ha^{-1}$ $yr^{-1}$ |
| Planted | Oct-12 | Dec-14 | Jan-15 |
| Land preparation | Burn, chemical strips/rows, thinning to stand density. No fertilisation. | Burn, chemical strips/rows, thinning to stand density. No fertilisation. | Burn, disking/spray, pitting/planting. No fertilisation. |
| Plant material | Natural regeneration | Natural regeneration | Seedlings PSO 11 |
| Spacing and stocking (stems per hectare) | 3 x 1.5 m<br>2222 SPH at establishment<br>1800 SPH at start of monitoring | 3 x 1.5 m<br>2222 SPH at establishment<br>1800 at start of monitoring | 3 x 2 m<br>1667 SPH at establishment and start of monitoring |
| Approximate average stand tree height at start of monitoring | 3.5 m | 2 m | 2 m |
| Spore trapping dates | 17 February 2016 - 07 April 2017 | 25 January 2016 – 07 April 2017 | 17 February 2016 - 07 April 2017 |
| Management activities during monitoring period | Weeded December 2016. | Pruned February 2017. | Weeded July 2016. Aerial application of Amistar Top (1 l ha-1), Decis Forte (50 ml ha-1) and BP Crop Oil (500 ml ha-1) to control rust and mirid 27 July 2016. |

Table 2 Results from the negative binomial GAM for *U. acaciae* teliospore abundance as a function of weather variables. GAMs have a parametric component and a smoothing part. $f(\cdot)$ = smooth term for a continuous variable, *SE* = standard error of the estimate, z = *z-statistic*, *P* = *P*-value, *edf* = estimated degrees of freedom, $\chi^2$ = *Chi-square statistic* and Dundonald is included as a reference group in the model. Significant values are denoted with P <0.05 = *, P <0.01 = **, P <0.001 = ***.

| Parametric coefficients[a] | *Estimate* | *SE* | *z* | *P* |
|---|---|---|---|---|
| Intercept | 3.72 | 0.08 | 45.70 | <0.001 *** |
| Site : Iswepe | -0.83 | 0.11 | -7.42 | <0.001 *** |
| Site : Moolman | -0.13 | 0.12 | -1.15 | 0.250 |
| Approx. significance of smooth terms[a] | | *edf* | $\chi^2$ | *P* |
| ***Average temperature*** | | | | |
| $f$(temperature) : Dundonald | | 1.25 | 3.45 | 0.052 |
| $f$(temperature) : Iswepe | | 0.00 | 0.00 | 0.404 |
| $f$(temperature) : Moolman | | 5.36 | 33.35 | <0.001 *** |
| ***Average relative humidity*** | | | | |
| $f$(humidity) : Dundonald | | 0.96 | 13.90 | <0.001 *** |
| $f$(humidity) : Iswepe | | 0.24 | 0.29 | 0.278 |
| $f$(humidity) : Moolman | | 3.23 | 24.46 | <0.001 *** |
| ***Average rainfall*** | | | | |
| $f$(rainfall) : Dundonald | | 2.81 | 18.67 | <0.001 *** |
| $f$(rainfall) : Iswepe | | 0.69 | 1.82 | 0.093 |
| $f$(rainfall) : Moolman | | 2.68 | 15.63 | <0.001 *** |
| Explained deviance | | | 31.3% | |

[a] The negative binomial GAM analysis of teliospore abundance as a function of weather variables (cf. equation (1)).

Table 3 Results from the negative binomial GAM for *U. acaciae* urediniospore abundance as a function of weather variables. GAMs have a parametric component and a smoothing part. $f(\cdot)$ = smooth term for a continuous variable, *SE* = standard error of the estimate, z = *z-statistic*, *P* = *P*-value, *edf* = estimated degrees of freedom, $\chi^2$ = *Chi-square statistic* and Dundonald is included as a reference group in the model. Significant values are denoted with P <0.05 = *, P <0.01 = **, P <0.001 = ***.

| Parametric coefficients[a] | *Estimate* | *SE* | *z* | *P* |
|---|---|---|---|---|
| Intercept | 1.88 | 0.08 | 23.78 | <0.001 *** |
| Site : Iswepe | -0.87 | 0.10 | -8.29 | <0.001 *** |
| Site : Moolman | -1.01 | 0.11 | -9.28 | <0.001 *** |
| Approx. significance of smooth terms[a] | | *edf* | $\chi^2$ | *P* |
| ***Average temperature*** | | | | |
| $f$(temperature) : Dundonald | | 4.72 | 52.13 | <0.001 *** |
| $f$(temperature) : Iswepe | | 0.00 | 0.00 | 0.633 |
| $f$(temperature) : Moolman | | 1.79 | 8.54 | <0.01 ** |
| ***Average relative humidity*** | | | | |
| $f$(humidity) : Dundonald | | 3.11 | 21.05 | <0.001 *** |
| $f$(humidity) : Iswepe | | 0.34 | 0.46 | 0.238 |
| $f$(humidity) : Moolman | | 2.24 | 8.55 | <0.01 ** |
| ***Average rainfall*** | | | | |
| $f$(rainfall) : Dundonald | | 2.80 | 19.78 | <0.001 *** |
| $f$(rainfall) : Iswepe | | 0.73 | 2.07 | 0.079 |
| $f$(rainfall) : Moolman | | 1.05 | 6.66 | <0.01 ** |
| Explained deviance | | | 45.2% | |

[a] The negative binomial GAM analysis of urediniospore abundance as a function of weather variables (cf. equation (1)).

## Figures


**Figure 1** (a) A spore trap, consisting of Vaseline coated slides on a wooden frame, deployed in plantations of *A. mearnsii* to investigate *Uromycladium* spore release timing. (b) Uredinia of *Uromycladium* on pinnules of *A. mearnsii*, with characteristic leaf yellowing (Reprinted by permission from Springer Nature: Springer, Australasian Plant Pathology (Uromycladium acaciae, the cause of a sudden, severe disease epidemic on Acacia mearnsii in South Africa, McTaggart et al.), [4895030941249] (2015)). (c-e) Characteristic telia of *U. acaciae*. Arrow (e) indicates a crust of teliospores extending above the telium. Crusts, consisting of a surface layer of teliospores that appear to protect the teliospores below from desiccation until the next period of wet weather, are present on all telia images.

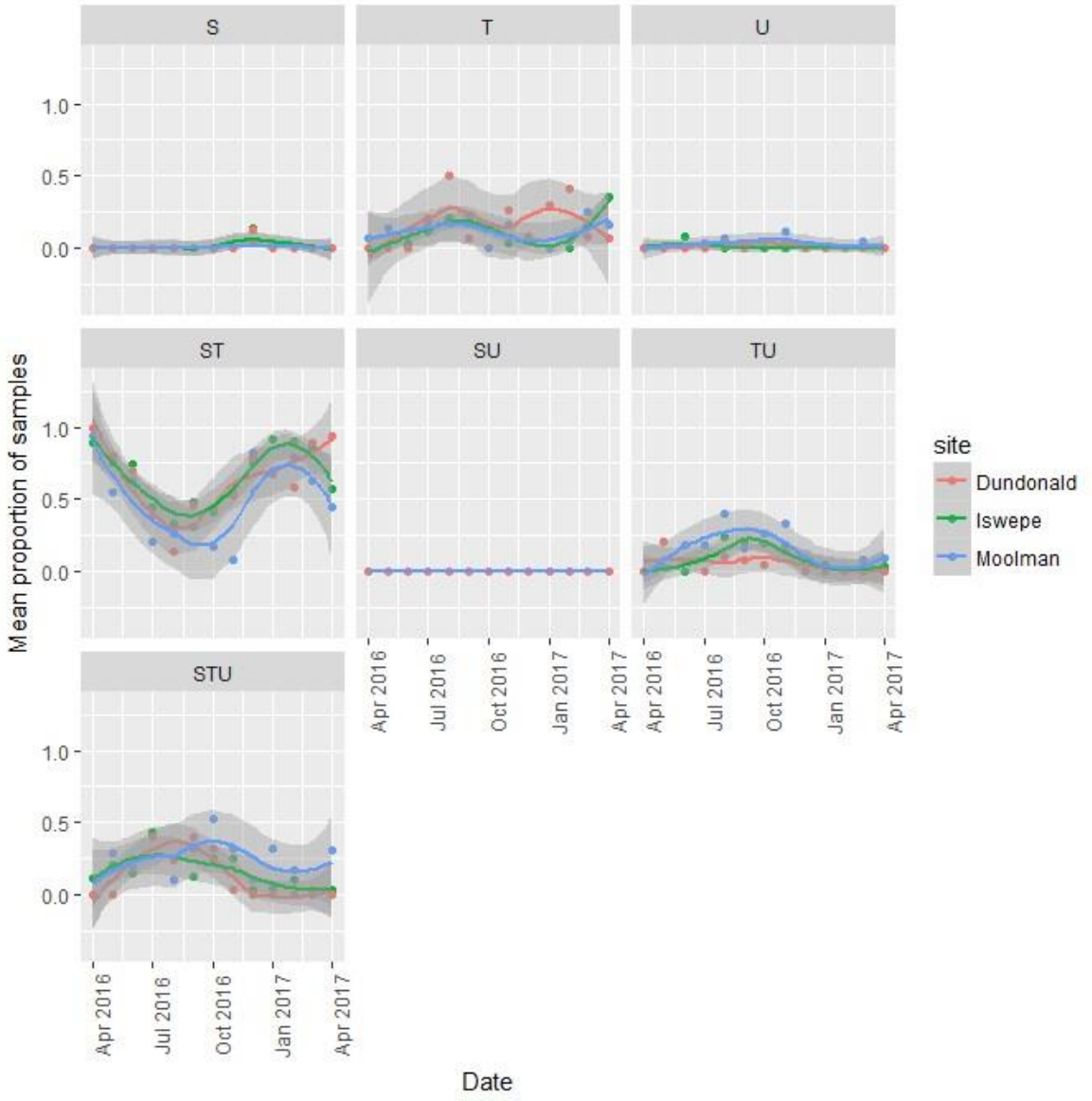


**Figure 2** Seasonal patterns of the presence and co-occurrence of different life stages of *Uromycladium* on samples of the most recent growth of *A. mearnsii* from three plantations. S, only spermogonia; T, only telia; U, only uredinia; ST, spermogonia and telia; SU, spermogonia and uredinia; TU, telia and uredinia; STU, spermogonia, telia and uredinia.

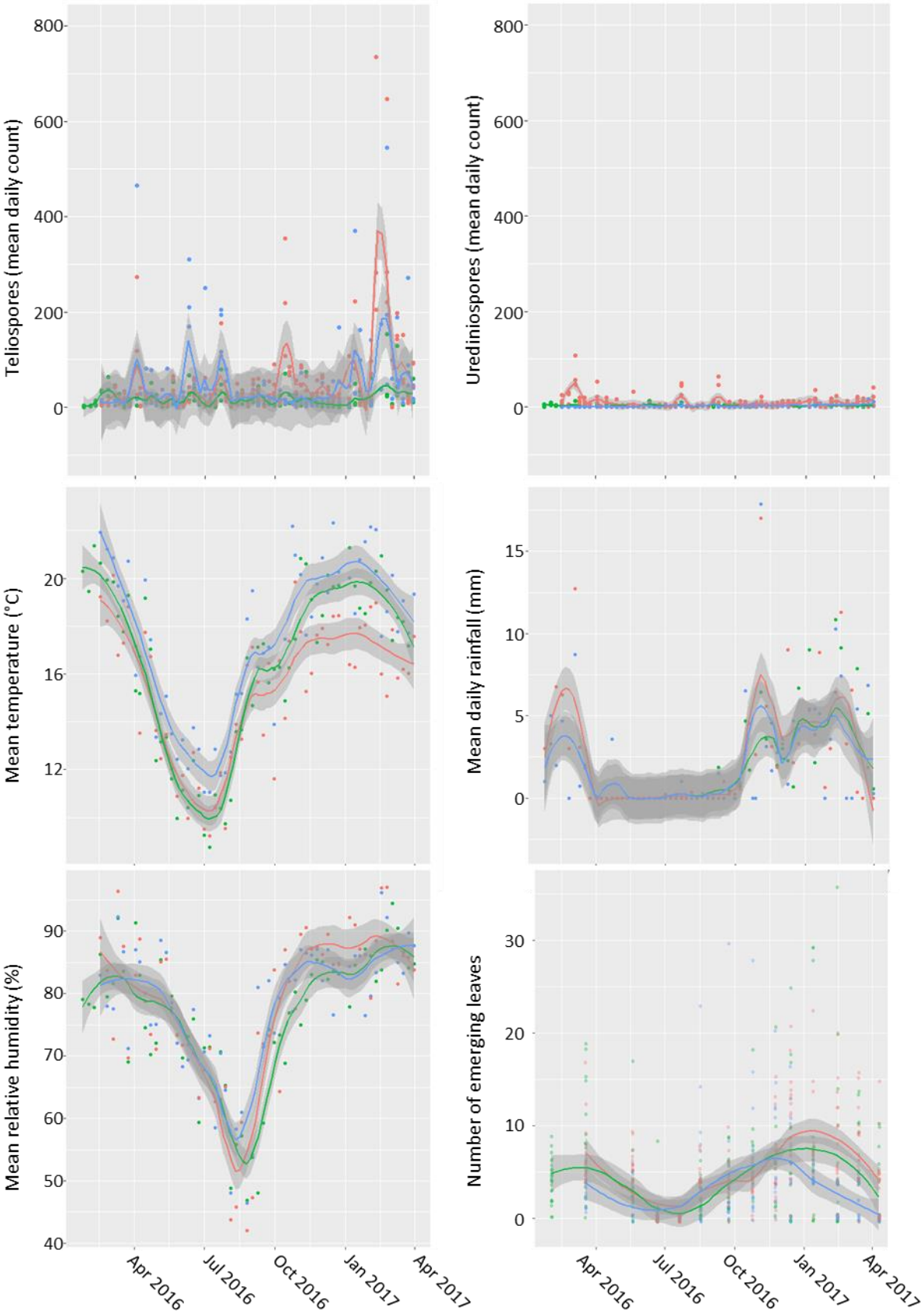


**Figure 3** Seasonal pattern of sporulation of *Uromycladium*, weather variables and phenology of *A. mearnsii* in three plantations. Red, Dundonald; green, Iswepe; blue, Moolman.

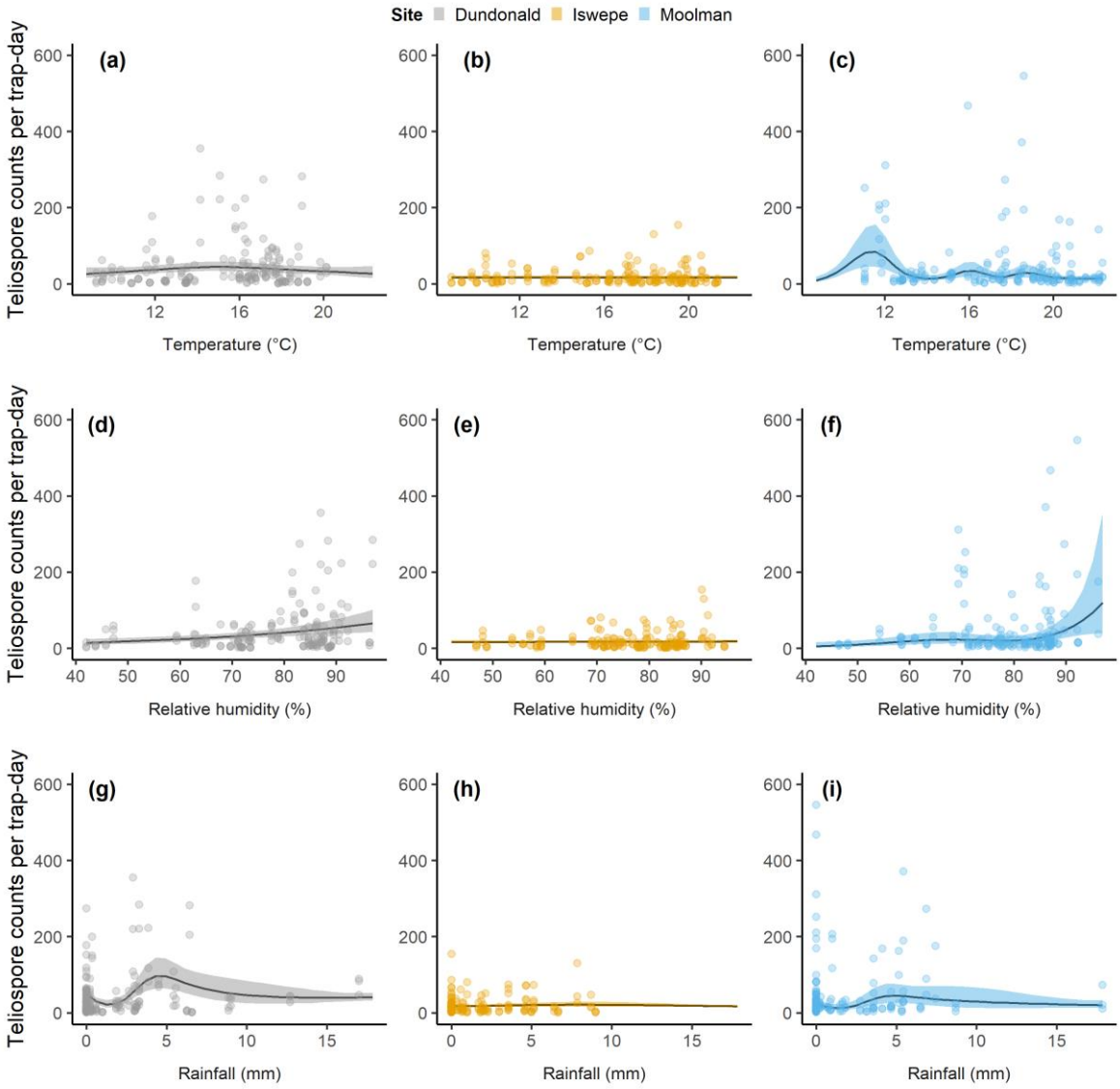


**Figure 4** Impact of weather variables on counts of teliospores of *U. acaciae* on spore traps in three plantations of *A. mearnsii*. Estimated smoothers for each weather variable for each site obtained from the negative binomial GAM (cf. equations (1)) applied on the teliospore data. The solid lines are the smoother and the shaded lines are 95% confidence intervals. Dots in each plot represent the observed teliospore counts. Values for weather variables are trapping period averages. Rainfall is average daily rainfall.

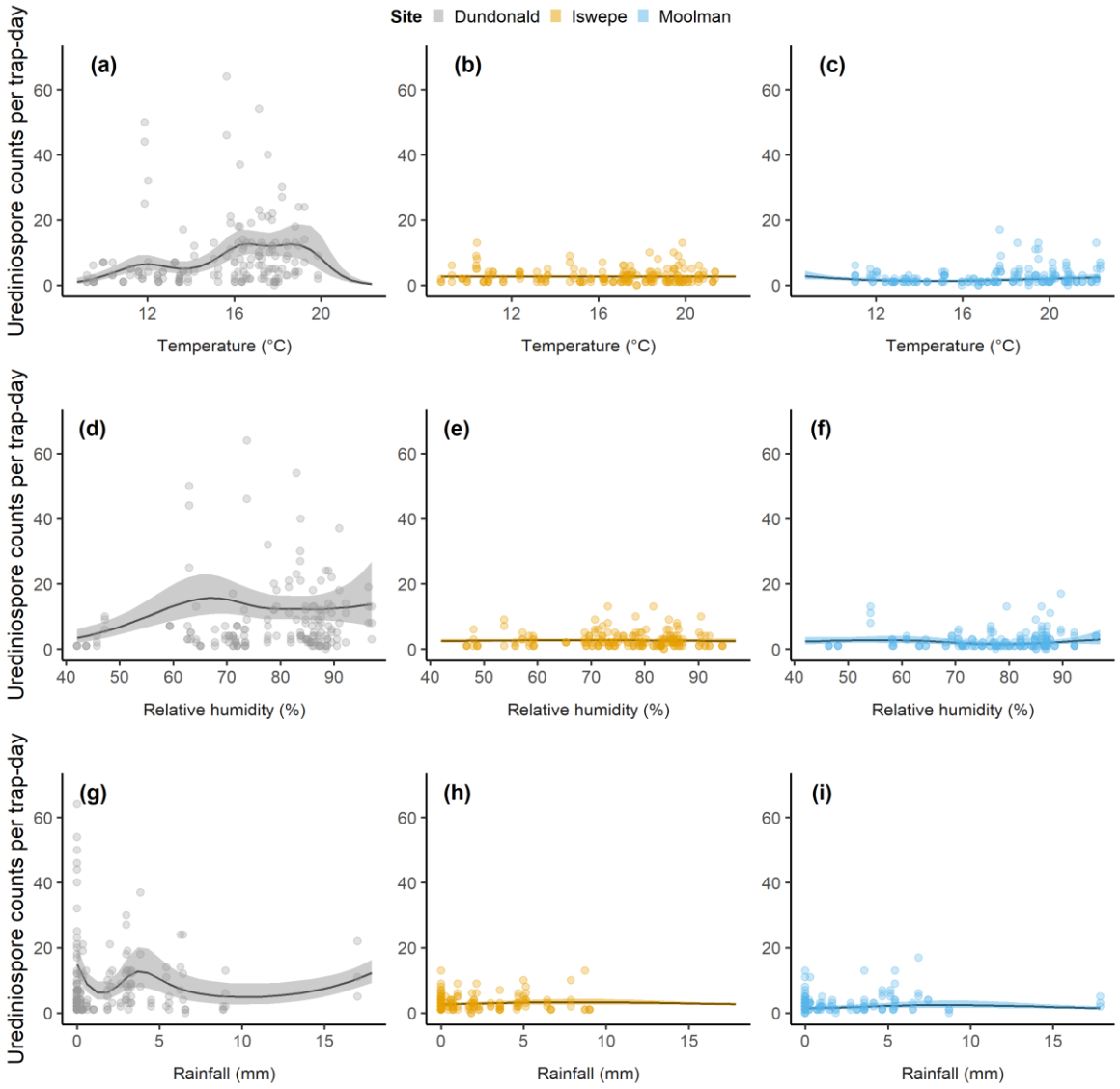


**Figure 5** Impact of weather variables on counts of urediniospores of *Uromycladium* on spore traps in three plantations of *A. mearnsii***.** Estimated smoothers for each weather variable for each site obtained from the negative binomial GAM (cf. equations (1)) applied on the urediniospore data. The solid lines are the smoother and the shaded lines are 95% confidence intervals. Values for weather variables are trapping period averages. Rainfall is average daily rainfall.


**Figure 6** Fixed SNPs and indel in the LSU and ITS regions of rDNA between uredinial and telial specimens of *Uromycladium* from *A. mearnsii* in South Africa.