Anthracnose *(Colletotrichum gloeosporioides)* development in a *Stylosanthes* spp. based pasture in response to fire and rain

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Abstract

Following a wildfire which caused patchy damage to a *Stylosanthes* spp. pasture in north Queensland, anthracnose development was monitored in the subsequent plant generation in unburned, partially burned and completely burned areas. The disease, caused by *Colletotrichum gloeosporioides*, appeared within four days after seedling emergence in unburned areas but not until after 14 days in both burned treatments, indicating that seedborne inocula did not establish an anthracnose epidemic. Although disease incidence increased rapidly in the unburned areas and had affected 70% of the plant population in the first 14 days, at the conclusion of the experiment (52 days after commencement) only 6.6% of the population in the completely burned area showed symptoms. There was no difference in anthracnose development between the two burning treatments. Laboratory isolations indicated that the fungus was unable to survive in sub-epidermal lesions remaining in plant debris exposed to the fire, and trap plants exposed daily during rainfall indicated very little inoculum was present in these areas. The bulk of the inoculum appeared to be that of a single, simple race of *C. gloeosporioides*. Provided other effects are considered, managing anthracnose in *Stylosanthes* spp. pastures and seed crops by fire is worthy of consideration.

Resumen

El desarrollo de anthracnosis en una pradera de Stylosanthes *spp. con parches dañados por un incendio, al norte de Queensland, fue registrado en la sub-siguiente regeneración de plantas en áreas no quemadas, parcialmente quemadas y completamente quemadas. La enfermedad, causada por* Colletotrichum gloeosporioides, *apareció a los cuatro días después de la emergencia de las plantillas en las áreas sin quemar pero después de 14 días en ambas áreas quemadas, lo que indica que el inóculo de las semillas no estableció una epidemia de anthracnosis. A pesar que la incidencia de la enfermedad se incrementó rápidamente en las áreas sin quemar y en los primeros 14 días la enfermedad había afectado 70% de la población, al concluir el experimento (52 días después del comienzo) únicamente 6.6% de la población de las áreas completamente quemadas mostraron síntomas de la enfermedad. No hubo diferencia en el desarrollo de la anthracnosis entre los dos tratamientos de quema. Aislamientos en el laboratorio indicaron que el hongo no pudo sobrevivir en las lesiones sub-epidérmicas que permanecieron en el rastrojo de las plantas expuestas al fuego, y las plantas controladas expuestas diariamente durante las lluvias indicaron que muy poco del inóculo estaba presente en estas áreas. La mayor parte del inóculo parece ser de una simple y única raza de* C. gloeosporioides. *Vale la pena considerar el manejo de la anthracnosis en pasturas de* Stylosanthes *spp. y en cultivos de semillas mediante el uso del fuego, siempre y cuando se consideren otros factores.*

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Introduction

Fire continues to be an important factor in the Australian environment (Kemp 1981). Pastoralists have traditionally used fire to modify extensive areas of pasture in northern Australia for various management reasons (Leigh and Noble 1981) although perhaps primarily as a means of removing rank, poor quality top growth and so increase animal access to new growth (Tothill 1971). Sporadic wildfires also frequently occur, particularly in areas where there has been a build up of combustible material following one or more wet seasons with average or above average rainfall. Pastures generally recover well from deliberate or accidental burning (Norman 1963), probably because the species have become adapted to a long history of exposure to fire (Gardner 1959).

Introduced legumes, particularly the perennial *Stylosanthes* spp. which are suited to large areas of northern Australian cattle pastures (Weston *et al.* 1981), vary in their susceptibility to burning. Gardner (1980) found that accessions of *S. guianensis* were least able to survive fires while accessions of *S. scabra* and *S. viscosa*, which are more suited to drier environments, tended to be more fire-tolerant. An experiment in north Queensland experienced a wildfire which killed two-year old stands of *S. hamata* cv. Verano and *S. scabra* cv. Fitzroy (Gilbert and Shaw 1980), while in Colombia, Lenne (1982) found mature plants of *S. capitata* survived fires in two successive years although regrowth after the second burn was slower. There also seems less chance of old plants of *Stylosanthes* spp. surviving fire (Gardner 1980; Mott 1982) and although seed can be destroyed, the heat can soften hard seed for early season germination (Mott 1982).

Improvement of pasture quality and sustainability through the widespread sowing of *Stylosanthes* spp. in northern Australia has been constrained to some extent by the fungal disease anthracnose caused by *Colletotrichum gloeosporioides* (Irwin *et al.* 1984). The environment in which these pastures are used, however, precludes the use of many disease control techniques employed conventionally in more intensive cropping systems. Fungus survival between seasons on diseased mature plants, seeds and plant debris provides a reservoir of inoculum for following regeneration. Such pathogen reservoirs may effectively be destroyed by burning (Hardison 1980; Luttrell 1989).

Gardner (1980) found anthracnose affected plants of *S. scabra* CPI 34925 were more susceptible to fire than healthy plants and postulated that burning may be a cheap and easy method of providing high quality, disease-free herbage in Australia. Experiments in Colombia with *S. capitata* showed that burning reduced anthracnose infections by up to 77% (Lenne 1982). The reduction was mainly due to the destruction of inoculum from severely diseased plants. Unsubstantiated claims of lower disease levels occurring in seed crops following burning of the previous season's crop residue have also been made by some seed producers of *Stylosanthes* spp. in Australia (J. Hopkinson, personal communication).

A wildfire occurred in an experimental area of *Stylosanthes* spp. based pasture in north Queensland in 1990. This paper reports the effects of the fire and subsequent rain on the regeneration of the *Stylosanthes* spp. with respect to the survival of *C. gloeosporioides* inoculum and anthracnose occurrence on the new plant populations.

Materials and methods

Design

A mixed accession *Stylosanthes* spp. and native pasture was established in 1975 on a 66 ha site near Mareeba (145°24′E and 16°58′S) in north Queensland (McKeague and Holmes 1979). On February 3, 1990 a wildfire of unknown origin passed through some of this area causing varying degrees of damage to the pasture which consisted mainly of *Themeda* spp., *Heteropogon* spp., *Stylosanthes scabra* and *S. hamata*.

Three distinct patterns of damage appeared in the experimental area following the wildfire. Substantial sections of the pasture were either killed, partially burned but containing live material, or untouched by the fire. These three patterns represented the field treatments and were contained in three replications throughout the pasture. Each block of treatments was restricted to an area 50 m × 50 m and data were collected from subplots established in each treatment.

Plots, 10 m in diameter, were marked within each treatment, and a 1.0 m^2 area was fenced with mesh to exclude grazing animals and rodents at the centre of each plot. Two other random points within each plot were located with stakes.

Data

At five random positions within each plot, stem debris of *Stylosanthes* spp. remaining either as standing material or surface litter was collected from a 0.25 m^2 quadrat. This material was washed in running tap water, cut longitudinally to expose any sub-surface anthracnose lesions and bulked according to treatment. The diseased pieces were surface sterilised using 0.01% mercuric chloride and washed in three changes of sterile water before 100 sections from the advancing margins of lesions from each treatment were plated onto oatmeal agar. Resulting colonies were identified after 10–20 days.

Twenty 5-cm long stem sections containing sub-epidermal lesions from each treatment were surface sterilised in mercuric chloride and washed as above. They were then placed in separate Petri dishes containing two moist filter paper discs and incubated at 25 °C for 20 days. The stem pieces were visually checked daily for indications of *C. gloeosporioides* colonisation.

Rainfall at the site was recorded daily. During rainfall events, 5 week-old plants of the *S. scabra* differential accessions Fitzroy, Seca, Q10042 and CPI 36260 were exposed to naturally occurring inoculum of *C. gloeosporioides* for 24 h periods within the fenced enclosures in each plot. One plant of each accession was grown in the glasshouse in a 100 mm pot which was removed to the site and contained in a hole to the depth of the pot. After exposure the pots were removed, enclosed in a moistened polyethylene bag and maintained at 25° ± 2 °C in a naturally lit glasshouse cabinet for 24 h. The bags were then removed and the plants incubated in the cabinet for a further 10 days before assessment for the presence of anthracnose.

Population counts of post-fire germinated seedlings of *Stylosanthes* spp. were recorded weekly over the first five weeks from a fixed 0.25 m^2 sub-plot at each of the two staked positions in each plot. The final (sixth) assessment was conducted 14 days later. Diseased seedlings were noted on each occasion for the 7 week period of the field observations (March 1, 1990–April 17, 1990).

Results

Eighty-seven percent of all isolations from sub-epidermal lesions found in unburned (UB) stems of *Stylosanthes* spp. yielded *C. gloeosporioides*. No fungi were isolated from similar lesions collected from burned stem pieces in either the completely burned (CB) or partially burned (PB) treatments. UB stem sections incubated in a moist atmosphere developed acervuli on the cut surface within 10 days. Acervuli also developed on two burned stem sections from a PB treatment while no fungus was apparent on any of the sections of stems from the CB treatment.

Rain occurred on March 1, 26 days after the wildfire and then on 15 additional days until the plant exposure experiments and field observations were completed on April 13 and April 17 respectively. No rain was recorded after 3 April (Figure 1).

Pots of trap plants were exposed on each of the 16 days when rainfall occurred. The highly susceptible cultivar Fitzroy, which does not differentiate between any of the known races of *C. gloeosporioides*, became infected in all the UB plots during the second rainfall event on March 2 (Figure 1). Anthracnose was subsequently recorded on Fitzroy plants from all UB plots exposed during each rainfall day except March 23. None of the Fitzroy plants exposed on this day (4 mm of rain) developed anthracnose after incubation.

Anthracnose developed on Fitzroy on only five occasions in the other two treatments (Figure 1). No disease was recorded on plants exposed in the PB treatment until March 22 and in the CB treatment until April 3.

No anthracnose developed on any Seca plants exposed in any treatment during the exposure period. Disease occurred on Q10042 and CPI 36260 plants in the UB treatments on two and five occasions, respectively, indicating at least two other races of the fungus, 4 and 4(a) were present (Chakraborty *et al.* 1988). These two accessions became diseased on one occasion only in the PB treatment and not at all in the CB areas.

Five days following the first rainfall (March 6), the seedling population of *Stylosanthes* spp. was determined in each treatment and no anthracnose lesions were observed on any of the cotyledons. There was no significant difference ($P > 0.05$) in the mean germination recorded in the 0.25 m^2 sub-plots. Mean subplot seedling numbers varied from 86.3 in the PB treatments to 165.7 in the CB treatments.

Anthracnose was first recorded in the seedling population in all the UB treatment sub-plots at the second field assessment on March 12 (Figure 2). The disease did not occur in either of the burned treatments until the third assessment, 3 weeks after the first rain (March 20). The percentage of anthracnose affected plants within the subplots was significantly ($P < 0.01$) lower in both burned treatments (2.3% and 0.2% for PB and CB treatments respectively) compared with the unburned population (70%). This trend continued and at the final assessment (April 17) 7

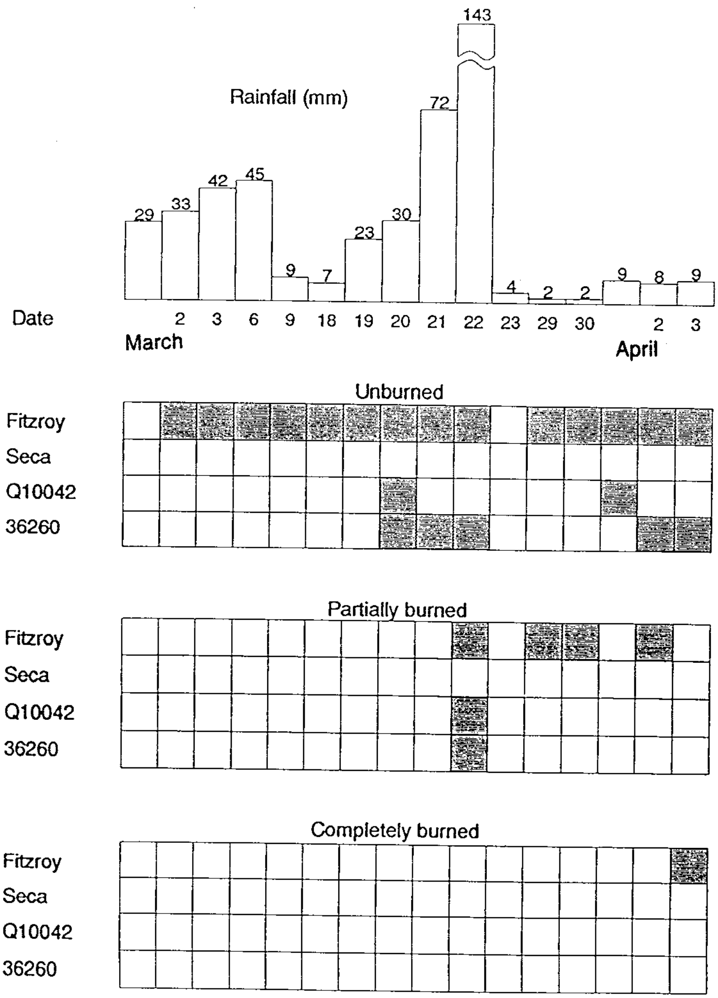

Figure 1. Anthracnose occurrence (shaded squares) in trap plants of four *Stylosanthes* accessions exposed in unburned, partially burned, and completely burned field plots during periods of rain between March 1 and April 3, 1990.

weeks after the first rain, 77.7% of the UB treatment population was affected with anthracnose compared with 13.8% of the PB, and 6.6% of the CB treatments' populations.

Discussion

Germination of *Stylosanthes* spp. occurred within 3 days of the first rainfall and disease symptoms became apparent 5 days later. Anthracnose symptoms normally appear within 5 to 7 days of inoculation of susceptible accessions in both the glasshouse (Davis *et al.* 1988) and the field (R.D. Davis, unpublished data). A high proportion of initial infections in this experiment, however, may be assumed to have resulted from seedborne infections (Davis 1987). This assumption is based on previous evidence indicating that the occurrence of anthracnose in field experiments is normally preceded by rainfall within the five to seven day period before symptoms appear (Davis *et al.* 1987). Symptoms recorded at the second assessment (March 12) could therefore have been initiated from a combination of seedborne and airborne inocula occurring during rainfall on March 6. The evidence from the Fitzroy plants exposed on March 6 indicates there was inoculum movement in the unburned treatment on that day.

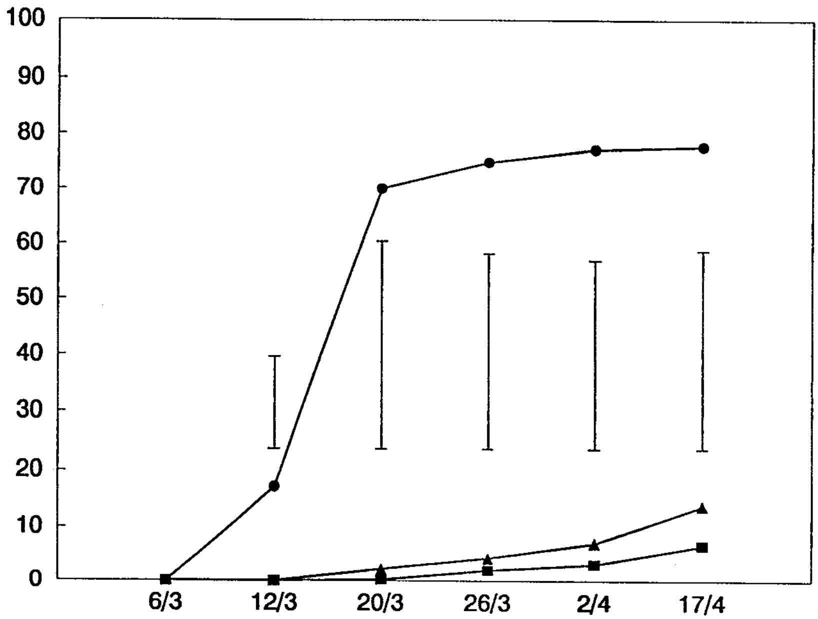

Figure 2. The effect of unburned (●), partially burned (▲) and completely burned (■) treatments on the incidence of anthracnose in new *Stylosanthes* spp. populations.

Vertical bars indicate l.s.d. at P = 0.05.

It had previously been found in laboratory experiments (Davis 1987) that successfully germinated infected seed produced seedlings with infected cotyledons before development of the first trifoliate leaf. Although it cannot be estimated how significant the level of seedborne infections was in the unburned treatment, it can reasonably be assumed from the data that seedborne inoculum was not a source for a disease epidemic in the completely burned treatment. Anthracnose did not occur in these plots to any extent until March 26, which was 23 days after seedlings had first appeared.

There was a large increase in the number of infections recorded in the unburned treatment at the third assessment (March 20). This rapid disease build up would have resulted from infections occurring between March 8–15. During this 8 day period, a total of 9 mm of rainfall was recorded over one day only (March 9). It can be speculated that this rain enabled these lesions to provide a large quantity of inoculum resulting in the disease increase recorded on March 20.

It is noteworthy that this inoculum from the unburned areas did not produce a similar level of disease in the burned treatments. This indicates a very restricted movement of inoculum from its production source. Even following the period March 19–23 when a cyclonic depression produced 272 mm of rain and windy conditions, only minor levels of disease were subsequently recorded in the burned treatments.

No infections occurred on the trap plants exposed on March 23 following five successive wet days. On March 22, 143 mm of rain fell over almost the entire day. It had previously been observed that fewer anthracnose infections occur during prolonged rain periods (Davis *et al.* 1987), although the reason has not been adequately established. Perhaps inoculum levels are depleted through continuous rain washing during such periods.

Inoculum of at least another two races of *C. gloeosporioides* was intercepted by trap plants of *S. scabra* Q10042 and CPI 36260 on March 20. It appears that there are very simple races or strains of the fungus predominating in this pasture.

Both burned treatments remained largely anthracnose free at the conclusion of this experiment, 48 days following germinating rain. There is good evidence to suggest that early infections due to seedborne inoculum were prevented by the fire, possibly by killing the fungus in the seed pods. Earlier work illustrated that most seedborne infections are found restricted to the pod (Davis 1987) and the heat generated by the fire may have been sufficient to destroy the pod infections without affecting seed viability.

There is also evidence that this disease, at least initially, may spread quite slowly. It is conceivable that if a large area of *Stylosanthes* spp. pasture growing in isolation is burned, anthracnose may take some time to re-establish in the area. Although it is not recommended to burn pastures solely to achieve this end, it may be cautiously added to the list of reasons for deliberately burning this type of pasture in northern Australia.

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