THE EFFECTS OF CEREAL STRAW RESIDUES ON GERMINATION, EMERGENCE AND PRODUCTIVITY OF ANNUAL MEDICS

by

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ABSTRACT

The studies reported in this thesis investigated the effects of cereal straw residues on plant density and dry matter production of annual medics (*Medicago* spp.). Straw residues, concentrated in walker rows, are known to be detrimental to medic productivity but there have been no previous reports quantifying these effects and little effort has been made to understand the relative importance of alternative biological mechanisms which could be responsible for retardation of medic growth.

To determine the form of the relationship between straw residues and annual medics, surveys were conducted in cereal stubble paddocks located on the Adelaide Plains and Yorke Peninsula Regions of South Australia. Increasing concentrations of straw were consistently associated with reductions in medic plant density. In most cases these plant densities were well below that considered desirable for optimum herbage production, consequently there were concomitant reductions in medic dry matter production per unit area. Comparison of alternative mathematical functions fitted to the data indicated that an exponential decay was most appropriate. Theoretical considerations of this particular function support it as the best choice.

Results from Field Experiment 1 in which seven straw concentrations were compared for their effects on medic emergence confirmed the findings from the surveys. Medic seed production, however, was generally not affected by the straw concentration. Field Experiment 2 showed that emergence of seedlings, from scarified seed, could be slowed by high concentrations of ground-straw mulch but mulch concentrations up to a maximum of 8000 kg/ha did not impair final seedling establishment. The field work provided indirect and direct evidence that high concentrations of straw residues were associated with increased intensity of pathogen attack of medic seedlings which resulted in depressed establishment of medics: however, the incidence of pathogen attack was erratic. Alternative means of managing straw residues are discussed.

Field Experiment 3 demonstrated that straw residues could substantially retard breakdown of medic hard-seededness over summer/autumn (the proportion of permeable

seed was 35 per cent less than that in an unmulched treatment) and this could exacerbate insulation effects caused by deep burial of medic seed in soil. This difference in permeability would almost certainly have been larger if the experiment had commenced earlier so its duration more closely approximated natural field conditions.

Large differences in the proportion of hard seed associated with concentrations of straw residues were also clearly shown when medic seed was collected from three field sites shortly before the seasonal break in 1986. It was concluded that the degree of thermal insulation of annual medic seed during summer/autumn is a major determinant of the density of naturally-regenerating medic seedlings. Long term effects of different thermal regimes on medic hard-seededness were detected similar to effects found on subterranean clover seed reported by other researchers.

Bioassay experiments were used to investigate possible allelopathic reactions by medic seedlings to leachates from cereal straw. Both the genotype of medic and of straw were important in determining the extent that radicle elongation was inhibited i.e. the typical seedling response. These effects were moderated by weathering of the straw or by microbial activity, either in the bioassay or where seedlings were grown in soil. There was little effect of leachates on the germination percentage of *M. truncatula* seed. Ecological implications of these results are discussed and phytotoxicity from straw residues is discounted as a factor contributing to differences in medic plant density as observed in the field investigations.

In pot experiments, increasing concentrations of macerated straw mulch were associated with a greater incidence of seedling mortality caused by *Pythium* fungi. In comparison, neither a plastic mulch nor addition of leachate from straw affected medic seedling establishment. It was concluded that the straw provided a source of nutrients which enabled rapid multiplication of the pathogen. Variation in microclimate and edaphic factors would account for the erratic frequency of pathogen attack on medic seedlings in the field.

STATEMENT

The studies presented in this thesis represent[5] original work carried out by myself except where due acknowledgement has been made in the text. This thesis has not been previously submitted in full or part to any other University for any degree or diploma. I consent to the thesis being made available for photocopying and loan if accepted for the award of the degree.

Paul E. Quigley

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GENERAL INTRODUCTION

1 GENERAL INTRODUCTION

Ley farming systems based on regenerating annual legumes, developed in southern Australia in the 1930's (Carter 1974; Macindoe 1975) but the sowing of medics and subterranean clover on extensive areas of the wheat belt occurred mainly after World War II; especially in the 1950's, coinciding with high wool prices. Pasture legumes are of fundamental importance for the success of these farming systems because they have the capacity to increase soil nitrogen levels through symbiotic nitrogen fixation. This cost-efficient development and maintenance of soil fertility is imperative for maximizing grain yields from cereals. The total dry matter yield of pasture herbage and its nutritive value both increase when legumes form a substantial component of the sward. Integration of legume based pasture and livestock production into rotations with cereals led to increases in cereal grain yields, reduced soil erosion, enabled higher stocking rates and stabilized farm incomes. Development of improved pastures also permitted expansion of farming into hitherto marginal production areas. The wheat-sheep zone derives its name from the two most common enterprises within it. Annual medics are well adapted to the soils and climate of large areas of this zone (Table 1.1).

The monetary value of pasture legumes to crop and livestock enterprises in Australia has been estimated as at least US$ 2500 million per year (Carter 1981). Further details of the place of annual legumes in farming systems and current management practices were described in the review by Puckridge and Carter (1980) and complementary discussions of ecological aspects of medics have been provided by Amor (1965), Quinlivan (1965b), Adem (1977) and Cocks (1980).

Table 1.1: Species of *Medicago* commonly found in south-eastern Australia.

Species	Adaption and cultivars
M. truncatula (Barrel medic)	The most economically significant species. Grows on alkaline soils with a high lime content and of medium to high fertility. Adapted to a Mediterranean climate with an annual rainfall of 250 mm or more. Current cultivars sown include **Jemalong, Paraggio, Ascot, Cyprus, Parabinga, Sephi** and **Borung**. The cultivar Hannaford was widely sown in the past but is now superseded.
M. littoralis (Strand medic)	Grows on alkaline soils and because of its shorter length of growing season it is adapted to lower rainfall areas. This species will perform satisfactorily on sandy textured soils but will not tolerate waterlogging. The cultivar **Harbinger** is sown.
M. polymorpha (Burr medic)	Adapted to soils rich in lime and of medium to high fertility where rainfall is winter dominant. Tolerant of waterlogging and moderately tolerant of high soil salinity. Predominantly occurs as non-commercial, naturalized ecotypes. Recently developed cultivars are **Circle Valley** and **Serena**.
M. minima (Wooly burr medic)	Widely found on less fertile and lighter textured soils; drought tolerant. Herbage production generally considerably less than commercial cultivars of other species. Occurs entirely as non-commercial ecotypes.
M. scutellata (Snail medic)	Grows on neutral to alkaline soils of either heavy or light texture. Both seed pods and young seedlings are susceptible to overgrazing by livestock. Regeneration may be erratic. Cultivar **Sava** is currently sown but un-named commercial ecotypes have been sown in the past.
M. rugosa (Gama medic)	Adapted to loam and clay loam alkaline soils and requires a minimum annual rainfall of 350 mm. A higher proportion of seed of this species becomes permeable over summer compared with other medics used in agriculture. Cultivars include **Paragosa, Paraponto** and **Sapo**.

It has been a widely held belief that after initial sowing of medic seed, levels of seed production in subsequent years, coupled with seed reserves in the soil, would be sufficient to ensure satisfactory regeneration. Consequently many farmers do not resow medics as a general practice. This belief has been disputed over the last decade in particular as medic plant densities in swards have frequently been noted as far below that required to ensure a satisfactory pasture (Carter 1981, 1982). Inadequate seed reserves in soil has been cited as a common, major cause of low medic density on farms (Carter 1980, 1981, 1982, 1986) and resowing has been advocated. The seriousness of the problems caused by low seed reserves cannot be overstated as it accounts for low quantity and inadequate quality in many pastures. Although numerous management and environmental factors have been attributed as causes of low seed quantities and poor pasture quality (Carter *et al.* 1982), few attempts have been made to compare the significance of different factors. This has serious repercussions as appropriate pasture management strategies, consistent with long-term, high production by medic- dominant pastures, cannot be developed without a more detailed knowledge of the basic ecology of medics. The low legume density in pastures and consequential poor nitrogen accretion is a major reason for the persistent trend towards increased use of nitrogenous fertilizers for cereal production. Data from the Australian Bureau of Statistics show that the application of mixed fertilizers (nitrogen and phosphorus combined) to wheat has doubled in Australia during the period 1981 to 1985 (151,000 to 303,900 tonnes) while the area cropped to wheat has been virtually static. Furthermore, in Victoria for example, where medics should be especially important components in rotation systems, use of these fertilizers has tripled to 14,600 tonnes.

Medic plant densities can be uneven within individual paddocks and this variability is often strongly associated with changes in the amounts of cereal straw over the paddock; low densities are especially obvious where walker rows of straw have been left after reaping. High concentrations of straw in these rows arise because the width of intake of straw at the front of the reaping machine is three to four times greater than the width of its rear exit chute. It is not known whether seed production by medic plants is reduced by large quantities of straw and whether this contributes to the wider problem of poor seed reserves. These observations have prompted detailed investigation of medic ecology described within

this thesis. As an example, photographs of heavy straw concentrations in walker rows and the impact on medic regeneration are shown in Plate 1.1.

Straw spreaders are now fitted to most modern harvesters but usually they only spread the straw over half to two-thirds of the machine width. This equipment is often inefficient as straw is left in heaps (Pratley and Cornish 1985) and the chaff component is unaffected in its distribution. Grazing by ruminant livestock is the most economical method of utilizing straw residues but use is generally restricted to a period of 60 to 90 days (Burns 1982). The straw and chaff of Australian cereal crops is about 45 to 50 per cent digestible, equivalent to dry summer pasture but with a greater likelihood of nitrogen deficiency (Allden 1980). The feed value is commonly enhanced by the presence of unharvested grain and green vegetation (either weeds or self-sown cereal) which livestock actively select (Mulholland *et al.* 1976). Initiation of grazing may depend on the type of livestock carried, the availability of feed from dried pasture and the amount of greenfeed in the stubble. The time of year that ewes are joined may also influence the grazing period. Breeding stock and young animals are generally given first access to stubbles (Allden 1980).

Examination of the literature describing the relationships between vegetative growth or reproduction and cover by straw residues revealed several alternatives, some of which could operate concurrently. Furthermore, these papers indicated that most of the relationships had been studied in isolation, consequently there were few attempts to examine them in an integrated system let alone one representative of a complex ecosystem within the wheat-sheep zone. In view of the insular nature of many previous investigations, it was considered essential that on-farm measurements be taken as part of this study. After deliberation on the potential magnitude of effects of specific mechanisms linking medic productivity with concentrations of straw residues it was decided to concentrate on factors discussed below.

PLATE 1.1: Straw walker rows and their impact on regeneration of annual medics.

Upper - A view of straw walker rows shortly after grain harvest of a barley crop, Port Wakefield 1983. The straw concentration may vary along the walker rows because grain reaping machinery has a tendency to drop the straw in heaps.

Middle - A dense straw walker row, Arthurton district 1983. The width of walker rows and the spacing between them varies from paddock to paddock according to the proportions of the reaping machinery used. In this case the width of straw within the row was approximately 1.2 m and the depth 30 cm.

Lower - Regeneration of annual medic showing the adverse effects of walker rows, Arthurton district 1983. Between walker rows, left side; within a row, right side. This is an example of the dramatic differences in medic densities which are frequently observed.

Brownlee and Scott (1974) and Scott and Brownlee (1974) found that the plant densities of cereals (sown at rates up to 33 kg/ha) had no effect on medic plant densities when medic was undersown but vegetative growth and seed production were both reduced. Competition from dense, self-sown cereals in walker rows was therefore discounted as a cause of gross changes in medic plant density, so this relationship has not been investigated in any detail here.

Incorporation of large quantities of straw into soil can reduce levels of available nitrogen and other nutrients including phosphorus and potassium (Lovett *et al.* 1982). These elements are utilized during periods of rapid increase in microbial biomass and therefore are less available for uptake by vascular plants. Depending on edaphic and environmental conditions, straw mixed into soil can assist in maintaining total soil nitrogen levels by attenuating nitrification (Mengel and Schmeer 1985) or cause an increase by providing a source of energy for free-living bacteria capable of nitrogen fixation (Halsall and Gibson 1986, Roper 1986). Immobilization of nutrients may restrict growth of annual legumes but this inhibition could not account for the gross differences seen in paddocks also symptoms of nutrient deficiencies are not apparent. Consequently this aspect was not included in research for this thesis.

Rice (1974) made an extensive study of nodulation by legumes and how this could be modified by compounds liberated from non-legume companion plants. Decaying grass residues reduced the haemoglobin content of nodules of white clover, and hence the nitrogen-fixation capacity, while with other legume species it reduced nodule number. It is conceivable that straw residues could exude similar compounds to those originating from these grasses and consequently may retard nitrogen fixation by annual medics. As the effects of this on medic plant density seemed unlikely to be large this line of research was not followed.

Cereal straw residues shade medic plants in the field. Growth rates of subterranean clover has been shown to be linearly related to irradiance levels, however this relationship is modified by both the leaf area index and the dry matter of the sward (Black 1963; Fukai and Silsbury 1977). Variation in shading is unlikely to be the reason for large differences in medic plant density observed in fields so irradiance levels were not investigated here.

The pattern of germination in the genus *Medicago*, in which only a small proportion of seed germinates at one time because of hard-seededness and hard seeds may remain viable over many years, has several ecological advantages. Firstly it minimizes the possibility of germination of large populations of seed following heavy rainfall during summer and early autumn when seedlings die through lack of moisture. In semi-arid environments where moisture availability often determines seedling development it is prejudicial to have all seeds germinate together with resultant severe competition for moisture. Should droughts occur and seed production be reduced, or precluded altogether, the seed bank in the soil ensures the possibility of regeneration in subsequent seasons. Secondly, the hard seeds remaining in the pods have further chance of being dispersed by animals either attached to fleece or via faeces after ingestion by the animals. This hard-seed character is most important for the successful dissemination and colonization of annual medics. Finally, when medic pastures are incorporated into rotational systems with field crops, the hard-seededness trait enables the medic seed population in the soil to survive periods of one, two or possibly three or more years of cropping when seed reserves are not replenished. Vegetative ground cover over summer is known to influence the breakdown of hard-seededness in annual legumes (Quinlivan and Millington 1962; Burton 1964) thus affecting the density of seedlings which regenerate in autumn. This relationship has not been researched in detail and quantitative data on the effects of shading by stubble on seed is scarce. This limited information suggests that straw residues are likely to have a major impact on medic density and therefore also on dry matter production. Consequently, changes in hard-seededness in medics have received major emphasis within this study.

Allelopathic effects from straw residues on subsequent crops have received considerable research attention overseas: however, these phytotoxic aspects are still imperfectly understood (Lovett *et al.* 1982). Research on potential phytotoxic effects from straw residues on regenerating annual legumes has been neglected in the past therefore this also became a major aspect of the work reported here.

The role of straw residues as a physical impediment to emerging seedlings and in the etiology of pathogen attack of young seedlings has also been investigated as both could be mechanisms responsible for reduced plant densities of medic.

Since the early 1980's there has been severe pressure on farmers in the wheat-sheep zone to improve their management so they may remain financially viable. There is considerable scope for enhancing the quality and quantity of annual medic-based pastures through changes in management and this would contribute to higher cereal-grain yields and greater productivity from livestock. More detailed information on the ecology the self-regenerating annual, legume-based pastures and the specific effects of straw residues will help farmers and advisers gain maximum productivity from these pastures.

2

LITERATURE REVIEW

2 LITERATURE REVIEW

2.1 Hard-seededness in annual pasture legumes

The contribution that hard-seededness makes to the agronomic success and agricultural importance of annual pasture legumes, especially medics, has been mentioned in the preceding General Introduction. Detailed description of the physiology of this characteristic and how it may be modified by environmental and biological factors is included below.

2.1.1 The nature of hard seed

A description of the development of terminology including "impermeable" and "hard" seed is given by Quinlivan (1971a). The *Leguminosae* has the greatest number of species with hard seeds but there are representatives of other families which also have this trait. Impervious seed coats must possess one or more layers consisting of tightly packed, thick-walled cells, with no stomata between them (Werker 1980). Even then, water would pass freely through the network of cellulose microfibrils of the cell walls unless water repellent substances are impregnated into the microfibril network and / or deposited upon it from within or without the cell wall. There is substantial disagreement on the layer of the seed coat responsible for impermeability of legumes (Quinlivan 1971a; McKee *et al.* 1977) and there have been virtually no attempts to quantify the various hydrophobic components in the seed testa (Rolston 1978).

Embryo dormancy is an alternative system of regulating germination. It is commonly found in *Trifolium* spp. and, although rare in annual medics, it does exists in some genotypes of *M. truncatula* (Quinlivan and Nicol 1971; Cocks *et al.* 1980). Quinlivan (1971a, 1971b, 1971c) holds that the most important germination-control mechanism in subterranean clover and annual medics is seed coat impermeability *vis a vis* embryo dormancy. However, Taylor (1972) attributed the protracted germination of barrel medic cv. Cyprus over late summer and autumn to embryo dormancy and he commented

that Quinlivan may have underestimated the importance of embryo dormancy as a germination- regulating mechanism in annual legumes.

Seed morphology

The outer layer of cells below the cuticle is known variously as macrosclerid, palisade, malphigian, epidermal or prism cells (Esau 1977). The macrosclerid cells are lignified and may contain tannin and pigments (Pitol 1935, cited in McKee *et al.* 1977). The outer end of these cells is pointed or domed and has characteristic thickening of the cell wall termed a cell cap. These suberized caps embedded in a pectic cuticular matrix make up the subcuticular layer which is covered externally by a waxy cuticle (Bhallah and Slattery 1984). One or sometimes two light lines (*linea lucida*) may be seen through the palisade layer. These result from diffraction of light and indicate a change in the chemical composition of the macrosclerid. In *Medicago* and *Trifolium*, the light lines result from the juxtaposition of suberin or cutin caps and the cellulose in the lower portion of the macrosclerid (Rolston 1978). This light line is found in all leguminous seed independent of hardness (Werker 1980).

Cells of the adjacent lower layer are variously termed osteosclerid, hourglass, "I", lagenosclerid, columnar, pillar, or spool cells (Esau 1977; McKee *et al.* 1977; Rolston 1978). They vary in size and shape but tend to be thick walled and loosely jointed with large air spaces between cells (Esau 1977). Generally there is only one row of osteosclerid cells but in a few species two or more rows have been observed (Corner 1951). Osteosclerids may contain pigment and tannins (McKee *et al.* 1977).

The third layer of the seed coat, generally less well defined than the macrosclerid layer has been variously termed, parenchyma cells, crushed parenchyma, nutrient cells, collapsed nutrient cells, mesophyll or spongy mesophyll cells. These may be pigmented and contain tannin or contain chloroplasts (Pammel 1899, cited by McKee *et al.* 1977). This layer has been suggested as a possible site responsible for impermeability (Bhallah and Slattery 1984). When ten genotypes of subterranean clover were compared, intense deposits of callose were found in this region in impermeable seeds but were absent in permeable seeds.

Between the parenchymatous layer and the endosperm an inner integument can be seen in some seeds. The outermost layer of cells in the endosperm, called the aleurone layer is often well defined. Another group of endosperm cells which are gelatinous with thick walls and contain no starch or little protein are believed to assist germination by swelling and generating pressure which ruptures the seed coat (McKee *et al.* 1977).

External features of the testa

The strophiole

The strophiole is an elevation on the seed coat on either side of a narrow, longitudinal depression, on the raphe between the hilum and the chalaza, typical of the legume subfamily Papilionoideae (Rolston 1978; Werker 1980). In structure it consists of an area where the macrosclerid cells are longer and narrower than elsewhere in the testa and on both sides of this structure, short macrosclerid cells are found overlying loosely-arranged cells (Hamly 1932; Aitken 1939; Hagon and Ballard 1970). Hence the strophiole is considered a primary site of weakness in the testa, prone to disturbance by mechanical action on the seed.

The hilum

The hilum is a scar on the seed coat left at the site of attachment of the seed to the ovary. At the hilum, in addition to the single layer of palisade cells belonging to the testa proper, there is an extra, inverted layer of counter palisade cells derived from the funiculus (Werker 1980).

The function of the hilum, reported in detail by Hyde (1954), is integral in the development of impermeability in the maturing legume seed. For *Trifolium* spp. and lupin he described its action thus: when relative humidity is low the counter palisade cells dry and shrink causing the fissure in the hilum to open thus permitting the seed to dry out but when humidity is high the counter palisade cells swell while the inner palisade cells remain relatively dry, thus closing the fissure and obstructing absorption of moisture. During seed-ripening the moisture content falls rapidly to approximately 25 per cent and thereafter it declines more slowly until the epidermis becomes impermeable at approximately 14 per cent moisture content. Further drying of the seed takes place only by diffusion of water

vapour through the hilum, consequently hard seeds tend to have a moisture content in equilibrium with the lowest relative humidity to which they have been exposed. Hyde found that the duration of the impermeable condition increased with the degree of desiccation. These observations support the conclusion of Aitken (1939) that the degree of dehydration of seed of subterranean clover determined the level of hard-seededness.

2.1.2 Physiology of breakdown of seed impermeability

Methods of artificial softening

Different artificial techniques for softening hard seeds have been reviewed by Porter (1949), Quinlivan (1971a) and Rolston (1978). The response to the different techniques vary considerably between genera. Mechanical scarification whereby seeds are rendered permeable by abrasion on rough surfaces is probably the most common commercial treatment (Rolston 1978). This technique is also frequently used in agronomic research to ensure that seed populations are permeable. Percussion, by vibrating seed against rough surfaces, has also been used in experiments to determine the site of least resistance in the seed coat.

Site in the testa

It was thought that the role of the testa in controlling permeability in legume seeds was simply due to suberin waterproofing. Provided that the testa was intact so water exclusion was complete, seed sensitivity was solely under control of the strophiole (Quinlivan 1968a; Hagon and Ballard 1970; Ballard 1973). Ballard (1976) working with *Medicago* and *Trifolium* species and using mechanical percussion claimed his results showed unequivocally that strophioles do not need to be acted on directly but perturbation of the testa at other sites may transmit stresses to the strophiole. The weakened strophiole becomes a point where water may penetrate, allowing imbibition to proceed. Indeed he found that direct mechanical action at the strophiole could be less effective than action elsewhere on the seed coat. The later results of Ballard suggest that the strophiole and the testa should be considered an integrated system; however, the strophiole remains the inbuilt point of weakness.

Quinone deposits on or in cell walls of the palisade or inner layers of the seed coat result from the action of catechol oxidase on phenols. These quinones have been considered as the cause of seed impermeability (Marbach and Mayer 1974; Werker *et al.* 1979; Werker 1980); however, the research of Slattery *et al.* (1982) with subterranean clover does not support this contention as in their work the development of impermeability in seeds was independent of oxidation of phenols.

The effect of depth of mechanical scarification on breakdown of hard-seededness has been investigated. Crown vetch (*Coronilla varia*) seed had to be penetrated to a depth of 98 µm or more for rapid imbibition of water and subsequent germination (McKee *et al.* 1977). At this depth, not only had the macrosclerid cells and other components of the outer integument been penetrated, but also the aleurone layer and even portions of the endosperm in some seeds. These findings support the suggestions of Ballard (1973) that the impermeable layer in the testa of subterranean clover persists well below the light line to levels deeper than was previously believed. Furthermore, Bhallah and Slattery (1984) have proposed that the parenchyma layer is responsible for impermeability.

Seed moisture content

The moisture status of the seed is an important factor which affects the degree of seed permeability (Aitken 1939; Hyde 1954). Recent pertinent research on this aspect has been largely confined to *Lupinus* spp. but the principles developed from this work are believed to be generally applicable. Quinlivan (1968b) found that seeds with a moisture content above 10 per cent had unstable impermeability and were able to absorb water slowly at random locations on the testa. Below about 8.5 per cent moisture the seeds were irreversibly hard. This later stage is the most common state for seed, as in a Mediterranean climate seed moisture content is generally of the order of 4 to 6 per cent or less (Ballard 1973). If seeds mature in relatively moist, cool conditions, seed moisture content may remain high enough for a proportion of the seed to be "semi-hard" (Gladstones 1958; Quinlivan 1968a; Quinlivan 1971a) but this type of environment is relatively rare.

When mature, hard seeds had been rendered permeable, Hagon and Ballard (1970) were able to show that exposure of these seeds to a relative humidity of 10 per cent or less could restore impermeability to 10 to 15 per cent of the seed. As these low levels of

humidity are rare events even in hot dry regions (Quinlivan 1971a), these observations are of only academic interest.

Sequential germination patterns

An ordered sequence of breakdown of impermeability of seeds within the pod has been found in a number of annual *Medicago* species (Kirchner and Andrew 1971; McComb and Andrews 1974; Ivory 1977). This occurs from the proximal (calyx) to the distal end of the pod but the mechanism responsible for this is not known (McComb and Andrews 1974). The gradation is not due to genetic polymorphism as these occur at random in seed. The sequential germination pattern can readily be observed in medic pods in the field when only one seed per pod usually germinates following rain in autumn.

Seed size influence

Investigations by McComb and Andrews (1974) of seven *Medicago* species showed that, within a pod, individual seed weight decreased from the calyx to the stylar end of the pod, while the moisture percentage of the seed showed no trend. In contrast Lebedeff (1947) and Quinlivan (1968b) found that seeds with a higher moisture content softened earliest. Gladstones (1967) found no correlation between seed size and hardness in subterranean clover seed.

2.1.3 Environmental and genetic effects

Alternating temperatures

Results from two laboratory experiments led Aitken (1939) to conclude that fluctuations in temperature were a major factor inducing hard seed of subterranean clover to soften. Two decades later, Donald (1959) contended that temperature fluctuations on the soil surface were responsible for seed softening in the field. A similar assumption was made by Andrew (1958) to explain softening of hard seeds of barrel medic over summer. However, it was Quinlivan (1961) who obtained data which showed clearly that temperature fluctuations, which followed a pattern similar to those experienced on the soil surface during summer, could increase seed permeability in *Medicago truncatula* . He investigated the pattern of seed softening in response to three temperature-fluctuation treatments, viz. 15-40, 15-60 and 15-75°C, by germinating seed samples at monthly

intervals. Permeability increased sharply for the first three months but thereafter remained unchanged and all temperature treatments affected seed permeability to the same degree. This research showed that in subterranean clover, in contrast to medics, the seed-softening effect was enhanced as the maximum temperature increased. Later research (Quinlivan 1968a; Bolland 1986) confirmed that medics tend to have a different pattern of hard seed decline when compared to other legume genera such as *Trifolium, Lupinus* and *Ornithopus* . With daily alternating temperatures Quinlivan (1968a) found that medics did not appear to show any increase in the rate of softening as the maximum temperature of the fluctuation increased from 50°C to 70°C. Over the six months of this experiment the relationship between hard seed content and time was essentially linear. He concluded that temperature fluctuations are the dominant factor in the breakdown of hard-seededness under field conditions and that the differences in the extent and pattern of softening for different strains within a species have substantial agronomic significance. Most importantly, the seed permeability characteristics of a genotype are of major importance when evaluating that plant type for any particular environment (Quinlivan 1961,1971b; Quinlivan and Millington 1962). In the field, few seeds soften over the winter, therefore seed reserves in the soil are maintained after the break of season (Carter 1981, 1982; Taylor *et al.* 1984).

Research on subterranean clover by Taylor (1981, 1984), suggests that there are two thermal mechanisms operating during the thermal induction of breakdown of hard-seededness. Weakening of the strophiole is especially dependant on the maximum temperature to which the seed is exposed. Final softening of the seed in response to fluctuating temperatures, probably involves physical expansion and contraction. Diurnally-fluctuating temperatures in the field are thought to combine both processes simultaneously. Taylor's data indicates that gradual rather than rapid temperature changes are more effective for seed softening whereby uniform rather than differential expansion and contraction of either the whole seed or some part of the strophiole is essential for fracture of the palisade cells at the strophiole. Differences between cultivars were most pronounced with relatively low temperatures.

Taylor *et al.* (1984), over a number of years, compared seed softening in a laboratory oven programmed with alternating temperatures of 15 and 60°C, with softening in the field. The ranking of cultivars of *T. subterraneum* for rate of seed softening was relatively consistent although there was an increased rate of softening in the field for six out of the eight cultivars examined. It was suggested that some unidentified factor/s other than temperature may be involved, possibly levels of ambient humidity in the soil.

Medic seeds extracted from pods of *M. littoralis*, *M. scutellata*, *M. tornata* and *M. truncatula* showed more softening than seeds left in the pods, when both groups were treated for nine months with a daily fluctuating temperature (15°-60°C), but there was no difference in *M. polymorpha* and *M. rugosa* (McComb and Andrews 1974). Differences between seed in pods and seed out of pods were attributed to insulation by the pod walls. A lower level of pod lignification in the latter two species may have reduced the insulation by the pod material thus the pods had no effect on breakdown of hard-seededness.

Hagon (1971) investigated hard seeds of subterranean clover and concluded that the effect of alternating temperatures was to make the testa permeable only at the strophiole. These findings supported similar observations for lupin where temperature fluctuations caused fracture at the strophiole alone (Quinlivan 1968b).

The influence of maturation conditions

In subterranean clover, hard-seededness is enhanced by slow maturation of the seed (Aitken 1939; Andrew 1956; Quinlivan and Millington 1962; Quinlivan 1965a, 1966) and if moisture stress occurs during seed maturation there will be a lower proportion of impermeable seeds. The rate of breakdown of hard seeds of subterranean clover is also related to moisture supply during seed maturation (Quinlivan 1971a). These conclusions were disputed by Taylor and Palmer (1979) who, using controlled environments found that a wide range of maturation conditions did not necessarily result in large differences in the degree of hard-seededness of Daliak subterranean clover. They suggested that site factors and differences in burr burial may have been confounded with seasonal conditions *per se* in previous investigations. However, Taylor *et al.* (1984) after collecting additional

data, adopted the view that adverse seed setting conditions may be associated with reductions in levels of hard-seededness.

Bolland (1986) obtained different patterns of seed softening depending on seed maturation conditions when a wide range of subterranean clovers and annual medics were examined. Differences in rate of hard seed breakdown between subterranean clover cultivars may be more important than the origin of the seed (i.e. growth conditions) for influencing the proportion of seed which is permeable according to Quinlivan (1966, 1968a).

Andrew (1956) showed that plants of barrel medic (*M. truncatula*) grown with adequate moisture and nutrients produced a higher proportion of hard seeds than plants grown under nutritional and moisture stress. Recent research, however, showed that the rate at which medic hard seeds softened was unaffected by the phosphorus regime in which the parent plants were grown (Bolland 1985). Generally, soil fertility appears to have slight or negligible effects on seed permeability (Quinlivan 1971a; Rolston 1978).

Collins (1978, 1981) working with subterranean clover has shown a significant Strain x Defoliation x Length of Growing Season interaction which influenced hard seed production. The timing of defoliation, whether before or after the onset of flowering, may determine the levels of hard-seededness. Taylor (1984) stated succinctly that environmental influences on hard seed formation and breakdown are inconsistent and poorly understood.

Genetics

Cultivars of subterranean clover can have vastly different softening responses to alternating temperatures either in the field or in the laboratory (Quinlivan 1968a; Taylor 1981, 1984). There are genetic differences between genotypes of the same species in levels of hard-seededness and rapidity of breakdown of hard-seededness and in some legumes mutant genes are known to cause totally soft-seeded strains (Lebedeff 1947; Donald 1959; Gladstones 1967; Kirchner and Andrew 1971; Quinlivan 1971a; Quinlivan and Francis 1971). Relatively few genes appear to be involved in hard seed inheritance (Rolston 1978) and genes determining initial hard-seededness are likely to be dominant (Slattery 1982). In *Lupinus*, *Vicia* and *Phaseolus*, one or two genes are responsible for control of hard-seededness (Lebedeff 1947; Forbes and Wells 1968; Gladstones 1970;

Donnelly *et al.* 1977). Pasture legumes, including subterranean clovers and annual medics, are also likely to have simple genetic control for this trait. According to Slattery (1982) there is greater potential for selection for rate of hard seed breakdown than for levels of initial hard-seededness .

Microbial effects

Microbial action has been suggested as a mechanism of softening seed (Raleigh 1930; Pfeiffer 1934; Trumble 1937; Aitken 1939; Mayer and Poljakoff-Mayber 1975) however Aitken is the only researcher to present relevant data and her results give no support to this hypothesis. Taylor (1984) found isolated pockets in the soil, especially between depths of 6-10 cm where dead subterranean clover seeds were recovered. These decomposed seeds had been subjected to massive fungal attack but the identification of the causal organism/s was not possible. Taylor inferred that fungal activity was responsible for some breakdown of hard seed in this case. It remains speculative whether microbial processes contribute in any way to breakdown of hard seed over long periods.

Influence of ground cover

In several strains of subterranean clover the rate of decline in hard seed content, over the summer months in the field, was accelerated as the magnitude of the daily temperature fluctuations increased, which was in turn, determined by the quantity of dry herbage remaining over the burrs (Quinlivan and Millington 1962). They suggested that reductions in the rates of decline in hard-seededness and a consequential low proportion of permeable seed in autumn were the probable reason for poor autumn germination in many legume pastures which had not been grazed over summer. Burton (1964) investigated the effects of density of cereal stubble on hard seed breakdown of existing seed (undetermined age) in the soil, at intervals over summer and early autumn, but his study was limited to one site and one summer/autumn period. In mid-autumn, numbers of permeable seed declined curvilinearly as straw concentration increased from 900 kg/ha to 8500 kg/ha; Burton's data and the curve fitted by regression analysis of his data during studies reported here are shown in Figure 2.1.

The relevance of ground cover by dried herbage, over summer and autumn, to rates of breakdown of hard seed has been ignored in a number of studies. Either the quantity of dry matter remaining on the soil surface at the end of the growing season has not been measured (Burton 1964; Quinlivan 1965a; Hagon 1974; Taylor 1985) or else the herbage has been removed altogether by herbicide application in spring or by cutting and removal in early summer (Taylor *et al.* 1984; Bolland and Collins 1987). As a consequence it is difficult to compare the results from these studies with other observations of hard seed breakdown in the field.

The location of seed within a soil profile could be expected to influence the rate of changes in permeability. Effects of depth of burial of seed in soil have been examined by Burton (1964) with *M. truncatula* and by Taylor (1984, 1985) with *T. subterraneum.* Taylor (1984) studied the effect of four depths of burial on the rate of seed softening in eight cultivars of subterranean clover over a three-year period. Seeds softened most rapidly on the soil surface and the rate of softening decreased significantly at 2 cm or more below the surface. These changes were closely associated with differences in soil temperature regimes as measured by thermistors. The only seedlings to emerge in the field came from burrs on the soil surface or no deeper than 2 cm. There was a highly significant Variety x Depth x Period of Burial interaction due mainly to differences in the second and third year. Taylor concluded that deep burial may maintain the viability of some genotypes for periods exceeding ten years. These results consistently demonstrate how the rate of seed softening declines with increasing depth of burial, to the extent that for relatively hard-seeded cultivars (Northam and Geraldton) there was negligible softening during three years of burial at depths between 6-10 cm. The medic results (Burton 1964) are of limited use as only one field site was examined over a single summer / autumn period and subsequent seedling emergence and establishment were not recorded.

In view of the differences in the pattern of seed softening for medics and subterranean clover the deficiency in our knowledge of distributions of medic seed in soil profiles and the relevance of this to hard seed breakdown is most serious. Some data has recently been obtained by Quigley *et al.* (1987) and these studies are continuing.

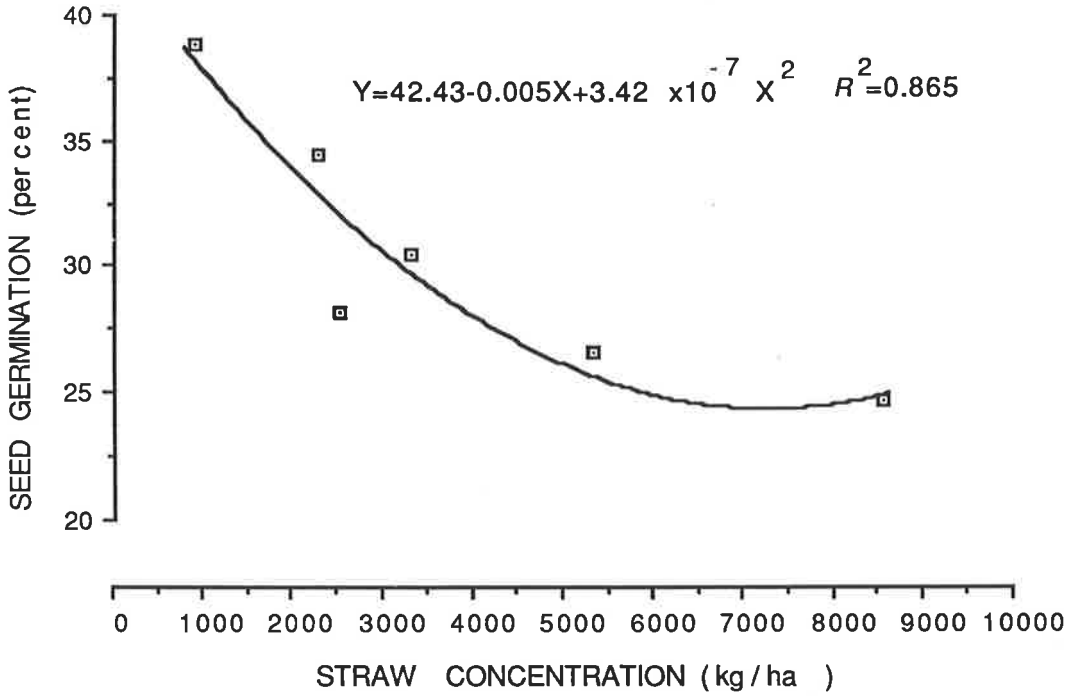


Figure 2.1: Seed germination of *Medicago truncatula* at the end of March after retrieval from the field where a range of straw concentrations covered the soil . (Figure plotted from the data of Burton 1964).

2.2 Effects of leachates from straw on plant growth and development

The term 'allelopathy' describes any directly- or indirectly-harmful effect by one plant (including microorganisms) on another through the production of chemical compounds (phytotoxins) that escape into the environment (Rice 1984). It must be considered distinct from competition where one plant reduces some growth factor such as light, water or nutrients to the detriment of some other plant in the same habitat. Furthermore, the term 'interference' should refer to the combined deleterious effects of one plant on another, that is, both allelopathy and competition (Muller 1969). Interference has been discussed in detail, including theoretical considerations, by Fuerst and Putnam (1983). The failure of many researchers to apportion allelopathic effects in competition studies has been noted in several reviews as a serious deficiency (Rice 1974, 1979; Putnam and Duke 1978; Putnam 1985).

There has been considerable research throughout the world concerning allelopathy over the last thirty years. With a greater knowledge of allelopathy many ecological phenomena such as vegetation patterning, plant succession and weed incidence in cropping systems are now more widely understood. Allelopathy has been recognized as a powerful ecological determinant, capable of altering the composition of many shrub plant communities (Harris and Kimber 1983). For example, leaf litter accumulating beneath *Eucalyptus baxteri* and *E. camaldulensis* can suppress the ingression of other plant species (del Moral and Muller 1970; del Moral *et al.* 1978). Rice (1985) believes that we are on the threshold of breeding crop plants that will inhibit growth of the chief weeds in a particular region by allelopathic action, hence reducing our dependence on herbicide control of weeds. This view, however, seems rather optimistic as there is very limited knowledge of genetic variation and genetic control of these chemical agents.

2.2.1 The phytotoxic effects of crop residues on plants

The framework of our knowledge of the phytotoxic impact of crop residues on subsequent plant growth and development has essentially been derived from research by two groups; one associated with T.M. McCalla at Nebraska, the other with Z.A. Patrick at

Toronto. Their studies have had a major impact so the progress of their research is briefly outlined below, together with relevant experimental contributions by contemporary or more recent workers.

In early laboratory studies, wheat mulch on the soil-surface inhibited germination of corn, while seed pre-treated with aqueous straw extract, and then germinated on agar, stimulated the growth of microorganisms which subsequently reduced seedling growth and caused roots to grow upwards (McCalla and Duley 1949).

Guenzi and McCalla (1962) studied the chemical nature and toxicity of inhibitors derived from a range of undecomposed plant residues including wheat and oat straw. All residues contained water-soluble phytotoxins which depressed germination and seedling development of corn, wheat and sorghum. The soluble salts and reducing sugars in these aqueous extracts of residues accounted for only a small proportion of the depression in seedling growth of test species, therefore the salt concentrations and osmotic potentials of the solutions were of minimal importance. They warned that the conclusions drawn from this laboratory research may not be closely related to results in the field because of the effects of environmental factors and residue management which were not taken into account in the laboratory studies.

Laboratory analyses by Guenzi and McCalla (1966a) identified and quantified the major phenolic constituents of unweathered, mature wheat and oat residues. All five compounds isolated, p-coumaric, syringic, vanillic, ferulic and *p*-hydroxybenzoic acids inhibited growth of wheat seedlings. These results are in agreement with those of Borner (1960) who also examined aqueous extracts from cereals. They suggested that in the field rather large amounts of these acids could be liberated in the immediate vicinity of the residues and that these could be sufficiently concentrated to inhibit growth of wheat seedlings.

Isolation and identification of phenolic acids in soils was accomplished by Whitehead (1964) and Guenzi and McCalla (1966b). As in cereal straw, the most common phenolic acids in soils were ferulic, *p*-coumaric, vanillic and *p*-hydroxybenzoic acids, commonly at concentrations of 0.01-0.1 mM in the soil solution. The concentration of the predominant phenolic, *p*-coumaric acid, was reduced when straw was incorporated into the

soil compared with stubble-mulched plots. Subsequent studies (Wang *et al.* 1967) identified and measured the concentration of these acids in a wide range of soils. When these concentrations were tested in nutrient culture solutions the growth of young wheat, corn and soybean plants was depressed. However, the concentration of various phenolic acids extracted from soil was found to be markedly influenced by the pH of the extractant solution used, with higher concentrations obtained at higher pH (Whitehead *et al.* 1981). Kaminsky and Muller (1978) recommend against the use of alkaline extractants if meaningful estimates of phenolics in soil are desired. Patterson (1981) has claimed that there is no correlation between the pH of solutions of phenolic acids, over the range pH 3.1-5.8, and their relative retardation of soybean growth.

Salient research by McCalla and co-workers examined differences in wheat, oat, corn and sorghum residues collected from the field (Guenzi *et al.* 1967). Water soluble extracts tested in a bioassay of germinating wheat showed the order of increasing toxicity was wheat, oats, corn and sorghum. Sequential sampling of the straw revealed that wheat and oats lost the toxic component in their residues after 8 weeks exposure under field conditions, but that corn and sorghum required 22 to 28 weeks for residues to become non-toxic. Leachates from straw from each of nine wheat cultivars caused variable responses in the bioassay. Leachate from different parts of mature plants were also compared in this study: however, a weakness in this paper was the failure to describe the climatic conditions during exposure of the residues in the field, so the degree of leaching by rain is unknown.

A thorough review of the research conducted by McCalla and his associates at the University of Nebraska has been published (McCalla and Norstadt 1974).

Following the incorporation of green vegetation into the soil, Patrick *et al.* (1963) examined samples of soil and decaying plant residues collected sequentially from the field. Phytotoxin production was assessed through the development of a technique whereby plant fragments were separated from soil and then a leachate was prepared using the plant debris alone. From their analyses they concluded that toxins derived from cold water extracts were most concentrated in the soil close to particles of decomposing plant material and that the toxins apparently did not move far from their point of origin. Of fundamental

importance, this research proved that phytotoxins could be produced under field conditions. Roots of plants grown in the field were especially sensitive and showed symptoms of damage such as discolored or sunken lesions, browning of the apical meristems and other injuries which ultimately resulted in stunted growth of seedlings. Phytotoxicity was most severe after barley, rye, wheat, sudan grass, vetch, broad bean and broccoli had been decomposing for 10-25 days. This confirmed earlier results (Patrick and Koch 1958) where toxicity developed relatively early in the decomposition process and decomposition in waterlogged environments induced greater phytotoxin development than decomposition under lower moisture regimes.

The review of Patrick *et al.* (1964) noted that plant residues were usually unevenly distributed on or in the soil and therefore many substances produced during decomposition were also unevenly distributed except when very large quantities of residue were present in which case relatively uniform damage was observed. They suggested that the most rational technique would be to separate out and examine the fragments of plant residue from the soil as these are the loci of toxin formation. Little phytotoxicity could be detected when aqueous solutions were extracted from soil and plant residues mixed together in proportions found in the field. Other results suggested that oxidation could remove or reduce phytotoxicity, so differences in aeration may account for variation in phytotoxicity from similar residues. They concluded that in these dynamic systems all effects are transitory; production, transformation and destruction of phytotoxic compounds occur simultaneously in the soil .

Additional experiments by this group showed that green plant material incorporated into soil in the field could liberate at least two identifiable phytotoxins, benzoic and phenylacetic acids (Toussoun *et al.* 1968). As these products were found to originate from barley, cotton, cowpea and soybean, the researchers proposed that these compounds commonly occur in nature when environmental factors are favourable.

Patrick (1971) while discussing soil aeration noted that roots, especially the primary root, were extremely sensitive to phytotoxic extracts. Although anaerobic conditions are conducive to the development of toxicity, Patrick believed that many investigators underestimated the potential for toxin production in non-waterlogged soils.

Citing the work of Greenwood (1961, 1968) and McLaren and Skujins (1968) he pointed out that fluctuations between aerobic and anaerobic states occur rapidly. Localized pockets of anaerobiosis frequently occur in the soil after rain. Environments conducive to production of high concentrations of phytotoxins may be more common than was hitherto realized and soils do not necessarily need to be waterlogged. Similar beliefs have been expounded by Parr and Papendick (1978) who stated that excessive amounts of plant residue may lead to depletion of O_2 and extended anaerobiosis under certain environmental conditions.

In Australia, Kimber (1967) found maximum inhibition of the growth of wheat and barley roots occurred after treatment with aqueous extracts from straw which had been decaying for 2 to 6 days. He also observed that roots were more sensitive than shoots to the toxins and that prolonged storage of straw or weathering in the field markedly reduced toxicity of extracts. At that time, field evidence to support the laboratory results was scant but subsequent research has confirmed many of Kimber's findings. Especially pertinent were experiments of Cochran *et al.* (1977) where aqueous extracts were prepared at weekly intervals from straw decaying in the field. Root elongation of wheat plants in a bioassay was inhibited but the extent of inhibition varied markedly over time suggesting cyclic development of phytotoxins in the field, similar to the fluctuations found by Kimber (1967). This work also demonstrated that the degree of root inhibition was linearly related to the concentration of leachates derived from straw. Plant responses in the field were not quantitatively examined by Cochran and his group.

Kimber (1973a) examined plant residues from a range of crop legumes, cereals and lucerne for their relative potential to develop phytotoxins during decomposition. Less mature plant material produced more of the toxic compounds than the unrotted straws. These unidentified inhibitors from straw were detoxified by microbial action during the rotting process which contrasts with results in his previous paper which indicated that some decomposition was necessary for development of toxins in wheat straw. He believed that variations in storage conditions may have accounted for these differences.

Kimber attempted to apportion the effects on seedling growth of nitrogen immobilization and toxin development when straw was incorporated into the soil or was

left on the soil surface (Kimber 1973b). Nitrogen fertilizer application could not entirely overcome weak growth of wheat, therefore he concluded that toxins must have been active in the soil. Contrary results have been obtained since by Elliott *et al.* (1981) who found no effect of straw on wheat growth in pots although the straw developed phytotoxicity according to bioassay results. Supporting evidence for Kimber has been provided by Bhowmik and Doll (1982, 1984) who could not overcome the inhibitory effects of residues by adding nitrogen fertilizer.

In a series of experiments Lovett and Jessop (1982) appraised the phytotoxic potential of residues from 12 crops, using wheat as the test species. When mature crop residues of wheat, lupin, peas, sorghum and rape were applied to the soil surface at a rate of 3 t/ha, wheat emergence in the field was reduced in all treatments. The length of the longest seminal root was shorter in wheat from treatments with applied residues than in residue-free controls, 18 days after sowing. Their observations corroborated previous conclusions that roots are especially susceptible to phytotoxins. They suggested that even though phytotoxic effects may be easily overlooked in the field, crops suffering toxic effects would be less fit to utilize or compete for environmental resources particularly under additional stress imposed by weeds, pests or disease.

Residues from mature, harvested crops, including sorghum, oilseed rape, sunflower, field pea, rye, oat, triticale and wheat exhibited selective inhibition of weed germination and growth under field conditions (de Almeida 1985; Purvis *et al.* 1985). In some treatments with crop residues however, weed infestation was worse which highlighted the differential effects on weed species and of crop residues. Barnes and Putnam (1983) examined the differences in weed inhibition caused by rye residues and a chemically inert, non-biological mulch. Rye reduced weed biomass by 63 percent relative to the control suggesting that allelopathy could contribute to weed suppression in the field.

2.2.2 The Nature of phytotoxins

Allelopathic properties have been attributed to an extensive range of organic compounds. Almost all crop residues appear to contain phytotoxic substances (Le Tourneau *et al.* 1956; Borner 1960; Nielsen *et al.* 1960; Patrick *et al.* 1963; McCalla and

Haskins 1964; Guenzi *et al.* 1967). Most phytotoxins are compounds termed secondary substances because they are of sporadic occurrence and do not appear to play a role in the basic metabolism of organisms (Rice 1974). Harris and Kimber (1983) have described the classes of compounds most commonly associated with allelopathic effects. Horsley (1977) who also described these chemicals, asserted that multiple classes of chemical inhibitors occur in many plant species. All too often the ease with which compounds can be isolated and identified determines the compound that is studied, rather than its importance in the effect under consideration. In addition the spatial distribution and rate of detoxification (either by microbial or physical processes) make it difficult to rank the relative contribution of different phytotoxins to any overall allelopathic effect. Additive and synergistic effects of phenolic phytotoxins have been studied in some detail (Rasmussen and Einhellig 1977, 1979; Einhellig *et al.* 1982; Blum *et al.* 1984, 1985a). These types of interaction between toxins further complicates any assessment of the relative importance of individual inhibitors.

Aromatic compounds

The compounds most commonly cited as phytotoxic are *p*-hydroxybenzoic, vanillic, *p*-coumaric and ferulic acids and these are frequently associated with retardation of root development (Toussoun *et al* . 1964; Moje 1966; Bell 1974; Rice 1974).

In view of the significance of phenolcarboxylic acids and their derivatives in allelopathy, a description of the synthesis, occurrence and ecological functions of these compounds in live plants is warranted. The shikimate biochemical pathway is the major metabolic route leading to the formation of aromatic compounds in living systems. It operates in microorganisms and higher plants, but not in animals, which is why the latter are dependant on dietary sources for the aromatic amino acids phenylalanine, tyrosine and tryptophan (Floss 1977). In addition to providing these essential protein constituents, the shikimate pathway is necessary for synthesis of essential cofactors and the important structural material lignin. It is also involved in the formation of a vast array of secondary metabolites. Deamination of phenylalanine and tyrosine leads to the simple phenylpropanes cinnamic and p-coumaric acids respectively (Whittaker and Freeny 1971). The benzene

ring may retain the 3, 4,5 substitution pattern of shikimic acid or this may be reduced as in ferulic acid, *p*-coumaric and cinnamic acids (see Figure 2.2).

Phenolic compounds rarely occur in the free form in living plant tissue being practically always present in the conjugated state (Harborne 1977; Horsley 1977). Most phenolics exist in leaf, stem or flowers in water-soluble, low molecular weight form as glycosides. The monosaccharides commonly associated with phenols include glucose, galactose, xylose, rhamose and arabinose. Glucuronic acid, a derivative of glucose, is also frequently bonded to phenols. One process which probably reduces the chance of phenolic compounds perturbing enzymatic reactions in the cell is polymerization. The two main types of phenolic polymers in plants are the lignins and tannins. Lignins are sequestered in the secondary layer of the cell wall, in close association with a cellulose matrix, where the phenolic hydroxyl groups may be hydrogen bonded to carbohydrate (Harborne 1977).

Regarding the ecological impact of these chemicals, Swain (1977) has noted that they are believed to form the basis of many plant-animal and plant-pathogen interactions by exerting attractant or deterrent activity. As an example, incorporation of phenolic acids into the cell wall increases its resistance to enzymatic hydrolysis which is usually a necessary step before the entry of fungal hyphae into the plant. Furthermore, in angiosperms, *p*-coumaric, caffeic and ferulic acids and their derivatives have been shown to act as deterrents to feeding by a wide variety of animals including weevils and locusts (Swain 1977). Allelopathic effects of one growing plant on another, mediated by exudation of chemicals, is another important ecological adaption. Comprehensive discussion of chemical defences of plants to pathogens and herbivores is provided by Levin (1976).

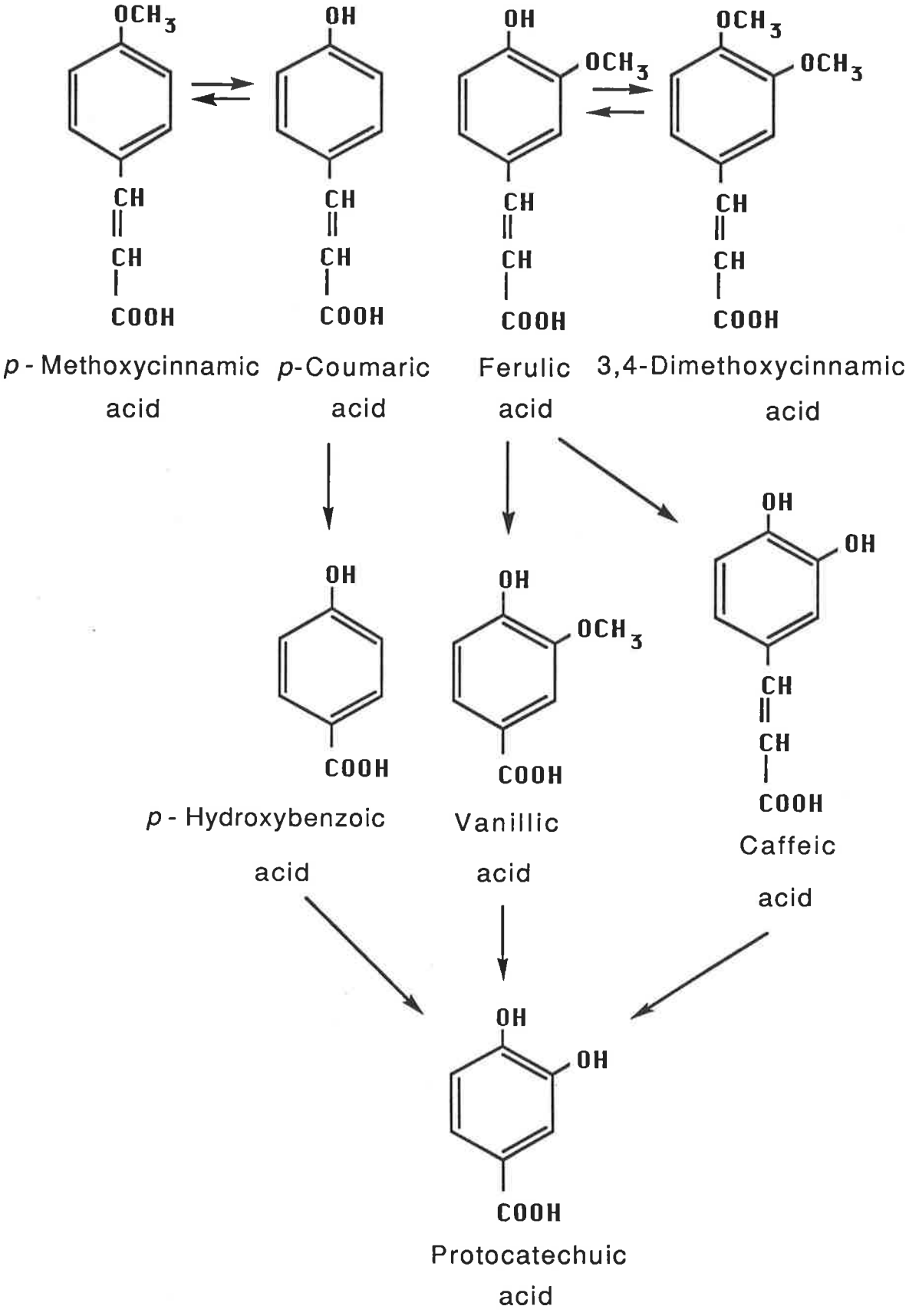


FIGURE 2.2: Probable decomposition pathways of common phytotoxic phenolic acids, after Hartley and Whitehead (1985).

Phenolic acids are sparingly soluble in water but may be readily extracted from soil by mild alkalis (Harris and Kimber 1983). They may be absorbed by roots of plants (Winter 1961) most likely by diffusion (Glass and Bohm 1971) and are transported in the xylem. Some phenolic compounds are toxic to plants and certain microorganisms if solution concentrations exceed 0.01 - 0.1 mM (Rice and Pancholy 1974; Glass 1976; McClure *et al.* 1978). Cell walls of graminaceous plants contain more phenolic compounds, especially ferulic acid and *p*-coumaric acid than do cell walls of most dicotyledonous plants (Hartley and Harris 1981). Many phenolic acids are photolabile, therefore bioassays should be conducted in the dark (Blum *et al.* 1984).

Antibiotics

Antibiotics are produced directly by fungi which may heavily colonize plant residues, especially straw close to the soil surface. Although some antibiotics may be phytotoxic, these compounds are strongly absorbed by clay minerals in soil so they quickly become less effective against growing plants (Harris and Kimber 1983). The antibiotic patulin, produced by the fungus *Penicillium urticae*, has been shown to cause damage to stubble-mulched corn and wheat (Guenzi and McCalla 1962; Behmer and McCalla 1963; McCalla and Haskins 1964; McCalla *et al.* 1964; Ellis and McCalla 1973). A major review covering allelopathic effects of this class of chemicals is given by McCalla and Haskins (1964).

Aliphatic acids

The aliphatic organic acids, which are common decomposition products of cell wall polysaccharides cellulose and hemicellulose (Lynch 1977; Harper and Lynch 1981) and from carbohydrates and proteins (Horsley 1977), may also have allelopathic effects. Normally only very small quantities of aliphatic acids are found in soils because under aerobic conditions heterotrophic bacteria and fungi utilize them as carbon sources (Horsley *ibid*). Consequently there have been few studies on the effects of these organic acids on plants under aerobic conditions (Whitehead 1963; Patrick 1971) although detailed studies have been conducted in anaerobic environments (Tang and Waiss 1978). While acetic acid

is one of the least toxic compounds in this group, it may still be important since it is produced in large quantities and under anaerobic conditions it may remain in the soil long enough to inhibit seed germination and plant growth (Lynch 1980; Putnam 1985). These compounds are more relevant to high rainfall regions where waterlogging is common. In the cereal growing areas of south eastern Australia they are unlikely to be of much practical importance because of the comparatively low rainfall.

2.2.3 Physiological responses of plants to phytotoxins

A comprehensive review of the known physiological responses of plants to phytochemicals with particular emphasis on phenolic compounds, has been presented by Horsley (1977). Among the effects elicited are inhibition of photosynthesis and respiration, perturbation of enzyme systems, protein and lipid synthesis and haemoglobin synthesis, also stomatal opening and membrane permeability can be disrupted. In view of the numerous reports of gross inhibition of root development, a more detailed discussion of the effects of phenolics on cell division, hormone-induced extension and mineral uptake by roots is included below.

Inhibition of cell division

A variety of allelochemicals have been reported to inhibit mitosis in plant roots (Rice 1974). Mitosis in onion and lily roots was completely halted by a solution of coumarin (Cornman 1946). Similarly, Jensen and Welbourne (1962) found mitosis to be reduced in root cells of *Pisum sativum* after treatment with *trans* -cinnamic acid.

Inhibition of extension growth

The plant growth hormones, indoleacetic acid (IAA) and the gibberellins (GA) regulate cell enlargement in plants. IAA is present in plants in both active and inactive forms and is inactivated by IAA oxidase (Putnam 1985). Several phenolic compound have been found to influence the activity of IAA oxidase; ferulic acid and 3,4-hydroxybenzoic acid are strong inhibitors while monophenolic acids such as *p*-hydroxybenzoic acid and *p*-coumaric acid are stimulatory (Zenk and Muller 1963; Lee and Skoog 1965; Tomaszewski and Thimann 1966, Machackova and Zmrhal 1976). The composition, concentration and distribution of phenolic compounds are complex and these compounds are metabolically

active, so it is difficult to determine the influence of any single phenolic compound on IAA metabolism *in vivo* (Lee 1980). Gibberellin-induced growth of pea seedlings has been inhibited by coumarin, cinnamic acid and several other phenolic compounds, but the tannins were more potent (Corcoran *et al.* 1972) .

Inhibition of mineral uptake

Various phytotoxins have rapid and pronounced effects on the appearance of roots of test plants (Rice 1979) and this has stimulated considerable research involving their role in mineral absorption (Rice 1974). Harper and Balke (1981) examined various phenolic compounds for their effect on K^+ uptake by excised oat roots. Ferulic and salicylic (*o*-hydroxybenzoic) acids caused 7 per cent and 15 per cent inhibition of K^+ uptake when the solution was buffered at pH 6.5 compared to 40 per cent and 64 per cent respectively in unbuffered solutions. They concluded that under certain pH conditions, phenolic acids such as salicyclic acid could significantly affect mineral absorption by plants in the field. Glass undertook a series of experiments to investigate the effects of phenolics compounds on the ion uptake by barley roots (Glass 1973,1974a,1974b; Glass and Dunlop 1974; Glass 1975). All phenolic compounds examined reduced ^{32}P-labelled phosphate uptake and K^+ uptake was retarded by 12 phenolic acids. Several benzoic and cinnamic acid derivatives rapidly depolarized the membrane potentials of barley root cells as well. Although he did not actually measure membrane permeability in any of these studies, Glass (1975) proposed that phytotoxins caused a generalized increase in membrane permeability which disrupted normal uptake of inorganic ions. Inhibition of phosphate absorption by soybeans, measured by McClure *et al.* (1978) corroborates the assertions of Glass.

In contrast, Bhowmik and Doll (1984) found that the mineral uptake and N and P nutrition of corn and soybean was independent of growth inhibition by phytotoxins. The inhibition or stimulation of N, P and K uptake was not consistent but depended on the residue source, residue placement in soil and soil texture. Addition of supplemental N and P to residue treatments provided no alleviating effect on retarded crop growth.

2.2.4 Enhanced pathogenesis caused by crop residues

In many allelopathic interactions it has not been shown whether plant growth is reduced by direct effects of phytotoxin or whether the chemicals condition the target plants to invasion by plant pathogens (Patrick *et al.*1963; Rice 1974). Early studies found that soil modified by the addition of salicylic aldehyde, a common breakdown product during plant decay, preconditioned roots of wheat and sugarcane to infection by root-rotting *Pythium* (Rands and Dopp 1938; Graham and Greenberg 1939). In the absence of the fungus, application of the chemical had little effect on plant growth and the chemical failed to stimulate growth of the fungus on culture media.

Cochrane (1948) suggested that root injury to young seedlings caused by water-soluble compounds derived from plant residues increased the incidence of root rot. Considerable evidence to support this proposition has been obtained by Patrick and Koch (1958), Patrick *et al.* (1963, 1964) who have concluded that the phytotoxins produced by decaying residues conditioned roots to invasion by low-grade pathogens.

Toussoun and Patrick (1963) found that exposure of bean roots (*Phaseolus vulgaris*) to extracts from decomposing plant residues, including barley, rye and wheat, greatly increased root rot caused by *Fusarium solani* f. *phaseoli*. Furthermore, germination of chlamydospores of this fungal pathogen was stimulated by substances derived from decaying plant residues (Toussoun *et al.* 1963, 1964) but this was attributed to nutrients liberated from decaying residues rather than to allelochemicals (Toussoun 1969).

Both susceptible and resistant varieties of tobacco and cotton became diseased by black root rot (*Thielaviopsis basicola*) when their roots were pretreated with toxins derived from decaying plant material (Patrick and Koch 1963; Linderman and Toussoun 1968a) and roots of cotton treated with phenylproprionic acid (a decay product) or barley extract were more susceptible to a nearly-avirulent clone of this fungus (Linderman and Toussoun 1968b).

Carley and Watson (1967) tested the effects of aqueous extracts from 23 crop residues on root development of wheat and radish seedlings in the laboratory and found that all extracts caused reductions in the root surface areas of the young seedlings and

increased root necrosis. They suggested that seedlings affected by phytotoxins have retarded water and nutrient uptake thereby becoming weak and susceptible to infection by pathogens. Apical necrosis and cortical degeneration affords an excellent opportunity for ingress of numerous saprophytic and mildly pathogenic organisms.

Singh and Pandey (1966) found soil amended with mature barley or oat straw increased the frequency of *Pythium aphanidermatum* but amendment with wheat straw reduced the population of this fungus. They speculated that not only the carbon to nitrogen ratio, but the overall chemical composition of the substrate and the nature of their decomposition products influenced their suitability as specific nutrients for the fungus. Possibly the specific microflora developing on such nutrients could be antagonistic to *Pythium* , hence various plant residues could suppress or stimulate *Pythium.* Garret (1970) suggested that root rot of strawberry, in which *Pythium* and *Rhizoctonia solani* were implicated, was induced by toxins derived from cover-crop residues.

A significant development occurred when Menzies and Gilbert (1967) demonstrated that plant residues on the soil surface could stimulate germination, respiration and growth of semi-dormant microorganisms (both fungi and bacteria) in a previously quiescent soil medium. These responses were elicited by water soluble, volatile substances liberated from various residues including wheat straw. Although similar stimulation had been detected many years before in agar culture, the authors believed that in the complex soil environment, that this would be an advantage to certain saprophytic fungi in colonizing plant residues.

Iswaran and Harris (1968), using pot culture, showed that the anaerobic decomposition of wheat straw previously incorporated into soil, could induce rapid growth of bacteria which were themselves highly toxic to seedlings of lucerne and subterranean clover. Additional evidence from glasshouse and field experiments has shown that the numbers of fungi and actinomycetes, although stimulated to some extent by greater soil water availability, were directly increased by crop residues which provided a nutrient and energy source (Doran 1980). The metabolic activity of all microbial groups in these studies increased with accumulation of crop residues on the soil surface.

Direct drilling of winter wheat through surface residue of the previous wheat crop increased damage by *Pythium* spp. (Cook *et al.* 1980; Cook and Hoaglund 1982). Moreover, chaff, which is less contaminated than most other plant parts with epiphytes and endophytes, is particularly effective in promoting *Pythium* attack on wheat roots (C. Chamswarng unpublished, cited by Cook and Baker 1983). With stubble mulching most of the population of microorganisms in the soil is often concentrated at the surface adjacent to the residues (McCalla and Army 1961) but decomposition products of lignin may differentially reduce spore germination and inhibit fungal growth (Lockwood and Filinow 1981). Inhibition or stimulation of pathogen populations depends heavily on the competition from other microbes so incorporation of residues into the soil may provide a biological control of pathogens in some circumstances (Sumner *et al.* 1981).

2.2.5 Variation in phytotoxicity caused by plant and environmental factors

It is well recognized that stress increases the build up of phenolic substances in plants. As applied stresses have been examined in only a limited range of plants, considerably more information is required before an understanding of the relationship between induced stress and allelopathy can be developed. For this reason, the general applicability of the conclusions in this section should be examined with caution.

Increased day length resulted in higher quantities of phenolics in plants (Tso *et al.* 1970) and increased exposure to ultra violet radiation is commonly associated with greater synthesis of phenolic compounds (Koeppe *et al.* 1969; del Morel 1972). According to Putnam (1985), light quality, duration and intensity may have substantial effect on regulation of synthesis of allelopathic compounds.

Deficiencies of boron, calcium, magnesium, nitrogen, phosphorus, potassium and sulphur have all been reported to enhance the concentration of phenolic compounds in a number of species of plants (Armstrong *et al.*1970; Lehman and Rice 1972; Koeppe *et al.*1976; Rice 1984). In contrast, magnesium or potassium deficiencies may reduce the levels of these compounds (Putnam 1985). Stowe and Osborn (1980) found that only plants which were suffering from nitrogen or phosphorus deprivation showed significant and consistent inhibition of growth by phenolics. Allelopathy should be most important in

soils of low fertility, or during periods of the year when fertility is low, particularly in arid regions where bacterial decomposition and leaching of allelopathic agents would be relatively slow. Water stress can cause substantial increases in the concentration of phytotoxic compounds in plants (Rice 1974). Where drought had been combined with nitrogen deficiency, sunflower plants had a 15-fold increase in phenolic compounds (del Moral 1972). Heat and chilling may also alter plant metabolism so that allelopathic compounds accumulate (Koeppe *et al.* 1970; Rice 1974; Steinsiek *et al.* 1982; Einhellig and Eckrich 1984), or plants become more susceptible to effects of exogenous phytotoxins. Research in the past has been inadequate, so these factors warrant a high priority for future research.

Stress imposed on plants by herbicides, invasion by pathogens, attack by insects, or uptake of external allelopathic chemicals may lead to internal synthesis of phenolic compounds (Woodhead 1981; Rice 1984).

Soil effects

Phenolic compounds in soil may be leached into deeper layers, metabolized by microorganisms, bound to soil mineral or organic components or incorporated into the humus fraction of soil following polymerization. Wang *et al.* (1967, 1971) and Kassim *et al.* (1981) used selective extractants to determine the site of phenolic compounds in soil then confirmed these findings by use of ^{14}C-labelled acids.

Differential adsorption of a range of phenolic acids by crystalline clay compounds and non-crystalline sesquioxidic minerals was examined by Shindo and Kawatsuka (1976) and Huang *et al.* (1977). Apart from protecting the phenolic compounds against microbial attack, the adsorption may contribute to oxidative polymerization by a catalytic effect, especially if montmorillonite is involved (Wang *et al.* 1978). Though phytotoxins may be chemically bound they may still retain phytotoxic activity (Rice 1984). Soils with a low adsorptive capacity allow rapid leaching of phenolic acids.

Microbiological degradation

A wide variety of microorganisms, including bacteria and fungi have the capacity to degrade phenolic compounds under aerobic conditions. Henderson and Farmer (1955) showed that the ability to metabolize p-hydroxybenzaldehyde, vanillin and ferulic acid was

very widespread among soil fungi. It should be noted, however, that many of the compounds produced during catabolism of plant-derived phytotoxins are themselves phytotoxic. Considerable research on phenolic acids, especially in relation to decomposition of lignin by microbes, has been reviewed by Dagley (1971). The effectiveness and rate of decomposition depends on an array of factors including soil type, soil physical properties such as moisture content, oxygen concentration and rate of diffusion, concentration of phenolics in the soil, intrinsic resistance of the compound to microbial attack and species of microorganisms (Moje 1966). Soil moisture may have paramount effect on the process as most microbes capable of degrading phenolic compounds are aerobic (Horsley 1977). Consequently, while the soil is anaerobic, phytotoxins may accumulate from decaying plant residues and may even be synthesized by microorganisms. The pathway of degradation for a wide range of phenolic substances has been described by Moje (1966) and Hartley and Whitehead (1985), see Figure 2.2. Listings of specific microorganisms capable of using carbon, in part or solely, from phenolics are provided by Black and Dix (1976) and Rice (1979).

Genetic effects

From the preceding sections of this review it is evident that plant response to phytotoxins varies considerably, depending on both plant species and the chemical nature of the toxin. Living plants or residues of mature annual plants also can be expected to have different capacities to produce allelochemicals. The variation between genotypes within a species has been neglected in the studies undertaken to date. Examination of differences in the response of genotypes to exogenous phytotoxins has been very limited indeed. The capacity to produce phytotoxic compounds has been studied in a restricted number of oat genotypes (Fay and Duke 1977) and in cucumbers (Putnam and Duke 1974; Lockerman and Putnam 1979), but the genetic basis of these differences has not been investigated. It may be expected that genetic control of this trait will be complicated in view of the wide range of chemicals with potential for phytotoxicity and the extensive influence of environmental conditions on the concentration and balance of these chemicals. Rice (1984) believes that there is an urgent need for research on the genetics of allelopathy.

2.3 Physical effects of crop residues on seedling development

Crop residue management significantly influences soil water content, soil temperature, soil strength, soil aeration and subsequent crop response. The technology of stubble mulching as a system of crop residue management has been continuously developing over the last 50 years in parts of the United States and Canada and to a lesser extent in Australia. The impetus for retention of crop residues has come from the need to control wind and water erosion of soil. The techniques developed in North America are applicable to Australian situations where similar erosion risks are important problems. Apart from controlling erosion, crop residues may also have considerable impact on the growth of weeds or desirable plants. The physical effects of crop residues on plant growth are discussed below.

2.3.1 Alteration of microclimate

When a mulch of protective material is used the force of falling raindrops on the surface of the soil is reduced (Ekern 1950; McCalla and Army 1961). This reduces compaction of the topmost soil layers and helps prevent formation of soil crusts which in certain soil types seriously hinders seedling emergence (Awadhwal and Thierstein 1985).

Where the soil surface is maintained in a permeable state by a mulch, the infiltration rate of rainfall is increased (McCalla and Army 1961; Black 1973; Russell 1973; Unger 1978; Schultz 1980). McCalla (1943) in emphasizing the importance of mulches for soil structure showed that a surface mulch was more important than soil organic matter content in increasing water infiltration. A major effect of mulches on poorly structured soils may be to increase the length of time anaerobic conditions persist in the soil, for it reduces the rate at which soil dries after rain, i.e. evaporation rate is less (Russell 1973) because both the amount of irradiant energy reaching the soil surface and the air movement immediately above the soil are decreased. Army *et al.* (1961) claimed to have had conclusive evidence that mulches only reduce evaporation from the soil surface as long as the surface remains wet. In semi-arid regions, mulch rates of 1 to 5 t/ac conserve considerably less moisture than heavier mulches (McCalla and Army 1961). Infrequent, light rainfalls minimize the beneficial effect of mulches with respect to moisture conservation. However, the

improvement of water storage in soils due to reduced evaporation may be especially important in areas where rainfall is unreliable at the optimum sowing time, as a mulch may retain sufficient seedbed moisture for seeding (Garland *et al.* 1976). Soil crusts have frequently been blamed for restricting gaseous exchange due to their low porosity and the presence of highly orientated soil particles which limit the supply of oxygen in the soil (Awadhwal and Thierstein 1985). While saturated, a soil crust can provide a very effective seal against diffusion but aeration usually improves at high moisture tensions when cracks develop in the crust. A model to predict potential evaporation from beneath surface residues was described by Van Doren and Allmaras (1978). Australian research indicates that when soil erosion is not a problem, stubble retention does not increase crop yields (Schultz 1980).

The principal effect of mulch on diurnal temperatures is to reduce the daily maximum temperature of a soil, especially under hot, dry conditions, without having any large effect on its minimum temperature (Russell 1973). The primary mechanism of this effect is the change in the radiant energy balance. Bright straw, as an example, may reflect up to 80 per cent more light energy than bare, dark soil (Van Doren and Allmaras 1978). As straw decomposes it becomes darker therefore less energy is reflected. Aase and Siddoway (1980) found stubble albedo to be 1.5 times greater than that of bare ground in autumn. This research also showed that total wind passage at 9 cm above the ground was 1.5 and 5 times greater over bare ground as compared with that over stubble 20 cm and 35 cm tall, respectively. Air turbulence near the soil surface is an important factor which influences soil temperature and evaporation. The soil temperature will be influenced by the soil moisture content as thermal conductivity rises with increased soil moisture content but the high specific heat of water reduces temperature fluctuations.

Aston and Fischer (1986), in New South Wales, have measured the effect of 2 to 4 t/ha of wheat stubble on soil temperatures, using bare soil (stubble burnt) as a control. Temperatures within the soil profile (down to 30 cm) were warmer during the day and cooler during the night where residues had been removed. This temperature pattern persisted both before and throughout the growing season. While large disparities in maximum temperatures were observed there were smaller differences in minimum

temperatures between the treatments. The improved early vigour of wheat sown into bare soil was attributed in part to the different patterns of soil-temperature fluctuations. From sowing to emergence the principal determinant of the rate of seedling development is mean daily soil temperature adjacent to the seed (Aston and Fischer 1986) and soil temperature near the growing point and region of leaf extension is the major factor which influences early leaf extension in grasses. Williams (1963) examined the effect of temperature on the emergence force and percentage emergence of seedlings of a range of forage legume species. Crimson clover had maximum emergence force at 20°C equivalent to 65 g but this dropped to 25 g at 15°C and 7 g at 10°C. There was little difference in the emergence force of alfalfa at 20°C and 15°C but it fell to about 3 g at 10°C. The detailed analysis of physical parameters contributing to control of soil temperatures (Van Doren and Allmaras 1978) contains a caveat that there are no accurate methods for predicting soil temperature changes caused by surface residues.

2.3.2 Mechanical impedance to emerging seedlings

Mulches of organic material including straw, hay and rice hulls have been widely used for weed control in horticultural production. These mulches prevent germination and/or emergence of weed seeds by increasing their apparent or physiological depth in the soil, by reducing the incident light, reducing oxygen diffusion and preventing diurnal temperature fluctuations which stimulate the germination of some seeds (Swarbrick 1981). Light may also be a stimulus for germination of many weed seeds (Shilling *et al.* 1985) therefore mulches may reduce germination. After emergence commences, seedling survival depends on initiation of photosynthesis before metabolic reserves are exhausted (Sedgley and Barley 1963), so mulches weaken seedling development by reducing photosynthesis (Robbins *et al.* 1942; Mercado 1979). The other major weed-control function of mulches according to Swarbrick (1981) is physical smothering which prevents emergence of the shoots.

High concentrations of crop residue left after grain harvest may resemble the mulches commonly utilized in horticulture, especially if particles of residue are more or less horizontal over the soil surface. As previously described, soil crusts may have

significant effects on the microclimate surrounding seedlings, but they may also physically impede seedling emergence. This has stimulated research, especially the measurement of maximum vertical thrust developed by seedlings (Arndt 1965a, 1965b; Jensen *et al.* 1972) Longer *et al.* 1986). Some studies have shown that small-seeded pasture legumes have median emergence forces highly correlated with seed size (Williams 1956) and that seedlings in a range of pasture legumes vary in the time taken to reach maximum emergence force, the time for which they maintain this force and the rate at which the emergence force dissipates (Jensen *et al.* 1972).

Lateral anchorage is essential for the shoot to exert its maximum potential thrust, so if a zone of low strength is immediately below a high strength layer, the shoot will tend to grow horizontally rather than vertically (Taylor 1971). Such diversion of seedling direction has been noted by Chambers (1962) and by Arndt (1965a, 1965b). Sedgley and Barley (1963) found that seedlings of *Vicia faba* could only withstand small axial pressure before bending if lateral support was poor and they concluded that a lack of lateral support could impair seedling emergence in the field. Investigation of how various levels of lateral anchorage affect seedling thrust needs more research (Taylor 1971). Other deficiencies in information include effects of environmental factors such as temperature, nutrition, water, aeration, phytotoxins, seed quality and diseases on seedling thrust exerted by various species. The interface between straw mulch and the soil surface offers an opportunity for seedling diversion as lateral stability of seedlings in this region is comparatively weak. As the hypocotyl increases in length its stem diameter diminishes and as a consequence the load-bearing potential of the hypocotyl declines markedly (Arndt 1965a), quite apart from the independent effect that the length of the stem has upon its tendency to bend. Data on cell size and shape for the cortical cells of pea and corn radicles indicated that while compression had only a slight effect on cell volume it caused a striking change in cell shape involving anomalous radial enlargement and reduced elongation, which was unlikely to result solely from the rheological behaviour of the wall under stress, and this suggested that growth regulators were involved (Barley 1976). Physical external forces on the seedling may possibly affect hormonal mechanisms which alter the vectors of force exerted by the seedling.

2.4 Discussion and conclusions

Hard-seedeness in annual pasture legumes that are grown in rotation with cereal crops is an important agronomic trait, especially for the farming systems used in Australia. As the fundamental physiological basis for hard-seededness has yet to be determined conclusively, detailed knowledge of seed physiological or biochemical responses to external stimuli which affect hard-seededness is limited and empirical associations have chiefly been studied in the past. Considerable research has examined aspects of hard-seededness in subterranean clover but annual medics, in contrast, have had less research attention. Medics generally have higher levels of seed coat impermeability compared with subterranean clovers and the patterns of change in hard-seededness are frequently different for the two genera.

Allelopathic effects of crop residues have been recognized for several decades due essentially to research conducted in Canada, the U.S.A. and Europe and interest seems to be increasing especially regarding the biochemistry involved. Although there are few centres in Australia which have participated in research on the subject and there are scant reports of investigations involving field work, general interest in allelopathy appears to be increasing here, stimulated no doubt by the steady accumulation of information and accelerated activity overseas.

Recently there has been increased commitment of plant pathologists to study diseases of annual pasture legumes (Taylor and Greenhalgh 1987; Johnstone and Barbetti 1987). Long term studies on this subject have been neglected in the past but detailed information is now being published. Because of the impact of environmental variables on the occurrence of these diseases, further research is imperative over a wider range of environments and it is important that current levels of research be maintained at least, so practical management strategies can be formulated which ameliorate the problems caused by diseases. These strategies are likely to take several years to develop based on experience with other plant disease problems.

3

THE EFFECTS OF CEREAL STRAW RESIDUES ON REGENERATION AND PRODUCTIVITY OF ANNUAL MEDIC PASTURES IN SOUTH AUSTRALIA

3 THE EFFECTS OF CEREAL STRAW RESIDUES ON REGENERATION AND PRODUCTIVITY OF ANNUAL MEDIC PASTURES IN SOUTH AUSTRALIA

3.1 Introduction

The potential deleterious effects that cereal crop residues have on regenerating annual legumes have been known for many years through widespread qualitative observations. However, the levels of retardation of pasture productivity caused by the presence of varying quantities of cereal stubble have been poorly quantified and the relative importance of basic, biological relationships underlying the effects of straw are undefined. Several examples can be found in the literature where warnings have been issued that straw, remaining from cereal stubble, ought to be reduced in quantity in order to optimize conditions for regeneration of annual pastures, either by grazing with livestock (Andrew 1958; Puckridge and Carter 1980; Puckridge and French 1983) or, as a last resort, by burning. Furthermore Webber *et al.* (1976, 1977) drew attention to straw walker rows left after the grain harvesting operation which they considered to be particularly troublesome as the excessive density of straw and other residue reduced both breakdown of hard-seededness and light available to young legume seedlings.

In spite of the visual impact of straw rows and the comments above, the lack of data detailing the effects of straw residues probably has been responsible for the omission of discussion of these effects in several recent major reviews on annual pastures, including those by Carter *et al.* (1982) and Puckridge and French (1983). However, a preliminary study was undertaken at Noarlunga by Carter and Pedler in 1982 (Pedler 1982) which formed the basis of the request to the Wool Research Trust Fund for funding this Ph.D. thesis research.

3.2 Field Survey 1: Arthurton district, Yorke Peninsula, 1983

The objective of this study was to quantify the deleterious effects of cereal straw residues on pasture productivity by measuring pasture parameters on paddocks under typical farmer management. Potential sites were selected on northern Yorke Peninsula where annual medics are the predominant legume in pasture swards. This district was particularly appropriate as there had been moderately good cereal growth during the previous growing season and consequently large volumes of stubble were present in some paddocks. Many of the alternative regions in South Australia had experienced severe drought conditions during 1982 , therefore the quantities of straw in these areas were extremely low and not suitable for the purposes of this survey. Topographical and other detailed environmental features of Yorke Peninsula, together with representative farming practices have been described by French *et al.* (1968).

The methodology adopted for the study was similar to several recent studies by other workers such as Adem (1977); Dahmane (1978); Carter (1981, 1982); Carter and Cochrane (1985) and Dear and Loveland (1985). A range of sites was selected where pasture management by farmers was typical of the district and measurements taken of seed reserves in the surface soil and subsequent pasture productivity. These techniques are especially useful as basic reasons for limitations to pasture yield can frequently be identified and the data is representative of the results which might be achieved under typical farming environments. These methods have not been previously used to examine the relationship between concentrations of cereal straw residues and pasture productivity.

This survey was intended to be a preliminary assessment of the effects of cereal residues as it was not possible to examine pasture development throughout the entire growing season.

3.2.1 Materials and Methods

A survey of typical farms in the Arthurton district, Yorke Peninsula, South Australia was commenced in October 1983. Eleven paddocks were selected where straw walker rows were evident and medic rather than clover was the predominant legume in the

pasture. Property owners were identified and all owners granted permission to undertake sampling on the areas of interest. Five paddocks were subsequently discarded from the program because hay cutting, cultivation or localised variability in pasture composition affected pasture productivity measurements. Locations for sampling within the paddocks were chosen at least 20 metres away from trees and diagonal headlands in the paddocks were avoided.

At each paddock, data were collected from two adjacent straw walker rows and two areas between walker rows. Five quadrats (40 x 40 cm), each separated by approximately five metres were taken from each strip, thus there was a total of twenty quadrats sampled at each paddock site. The widths of the sampled walker rows and adjacent strips were measured.

The numbers of medic plants growing in each quadrat were counted and then all herbage and straw was removed from a quadrat by cutting to ground level with knives. After transportation the samples were stored in a coolroom at 2°C. Herbage components were handsorted from the straw then soil and gravel contaminants were removed from the straw by hand sieving. Herbage and straw were dried in a forced-draught dehydrator at 80°C and weighed.

Two cylindrical samples of soil, 5 cm deep, were extracted from each quadrat area using a Coile soil corer with a diameter of 106 mm (Coile 1936). These soil samples were air dried, crushed and passed through a 2.0 mm aperture sieve so that medic seed collected on the lower 0.5 mm aperture sieve, however a larger top sieve was used if snail medic seed was present. Seeds and fragments of organic matter were separated from the remaining soil by a flotation process using the organic solvent perchloroethylene (Perclean®, ICI Australia Limited, Melbourne) as described by Carter *et al.* (1977). Medic and cluster clover (*Trifolium glomeratum*) seeds were hand-sorted from the debris, separated into species and then counted. *M. polymorpha* was not separated from *M. truncatula*, but this introduced little error. Seed reserves in the top 5 cm of soil are expressed on an area basis.

Seed production during the growing season was measured in early December. Dried herbage and pods were collected from within 28.8 cm diameter steel rings (5 per row)

avoiding areas where herbage had been cut previously. Soil and excess herbage were removed by sieving then pods were handsorted into species and weighed. Pods were manually threshed by rubbing them between corrugated rubber sheets, after which seed was cleaned by further sieving then weighed.

The percentage reduction in medic herbage production within the walker rows was calculated relative to the yield between rows. Furthermore, the total percentage reduction in medic herbage yield, on a paddock basis, was derived as a product of the loss in the rows (described above) and the proportion of paddock area covered by the walker rows. Photographs showing the sampling methods used in the field, are presented in Plate 3.1.

Statistical analyses

For each pasture component viz. medic, green cereal, grass, broadleaf weed, and cereal straw, mean dry matter per unit area in the walker rows was compared with that between rows using an unpaired *t* -test. A large range of dry matter yields made it necessary to first transform the data to log(x) or, where zero yields occurred log(x+1). Medic plant densities were similarly analysed. It was not necessary to transform seed densities before the *t* -tests were calculated.

Two models were fitted by regression in an attempt to quantify the relationship between straw density and medic plant density or medic dry matter production. In the first model green cereal dry matter was also included as an independent variable.

Model 1: first order polynomial $Y=A+BX+CZ$

Y is medic dry matter (kg/ha)

A is a constant

B,C are coefficients

X is straw dry matter (kg/ha)

Z is green cereal dry matter (kg/ha)

Model 2: exponential decay $Y=Ae^{BX}$

Y is medic dry matter (kg/ha)

A is a constant

B is a coefficient

X is straw dry matter (kg/ha)

PLATE 3.1: Field Survey 1 - Techniques and equipment used for sampling in the field

TOP - Cutting herbage and straw samples from within a quadrat

MIDDLE - Extraction of soil cores with a Coile corer. Seeds were later extracted by sieving, then they were counted

BOTTOM - Medic seed production was measured by collecting pods from within steel rings at the end of the growing season

3.2.2 Results

The quantities of each vegetative component of the pasture sward together with the straw concentrations at each site are shown in Table 3.1. There was a consistently large reduction in mean dry matter production from medic plants growing in straw walker rows compared with medic production between the rows. The vegetative growth of broadleaf weeds was similarly reduced in the walker rows. The chief reason for the relatively low quantities of medic herbage produced in the walker rows was the reduced densities of medic plants in these strips (Table 3.2). In this survey the mean density of medic plants between the walker rows was always at least twice as high as the mean medic plant density in the walker rows and at some sites exceeded the mean density in the rows by a factor close to ten fold.

The quantities of straw present did not vary greatly from paddock to paddock but herbage production did vary considerably (Table3.1). This range in medic herbage production between sites was largely the consequence of differences in seed reserves in the top 5.0 cm of soil (Table 3.2). The density of medic plants growing between the walker rows, expressed as a percentage of the "initial" seed reserve at the commencement of the growing season (i.e. the sum of the seed reserve and plant density measured during the growing season) had a narrow range viz. 19.5 to 26.7 per cent, except for Site 2 which was 5.9 per cent. The predominant medic species present at Site 2, *M. scutellata*, was absent from other sites and this may be partly responsible for the deviation from an otherwise consistent pattern. Alternatively, the prior history of the seed at Site 2 may account for a higher intrinsic level of hard-seededness, especially if the seed reserve was particularly old, or some environmental factor may have reduced seedling establishment.

In all cases there was more herbage production by self-sown cereal plants in the walker rows undoubtedly due to concentration of grain in these strips during grain harvest operations.

At the end of the growing season, the mean yield of medic seed produced in the walker rows differed little from that between the rows (Table 3.3). Those plants established between the walker rows made lateral growth which partly covered the straw rows and subsequent seed production on this lateral growth would explain why there was an even

pattern of seed production in each paddock despite fewer medic plants in walker rows. Low density of medic plants and variability in their spatial distribution at Sites 2 and 3 were the likely causes of the significant difference in the mean seed densities of medic. The large quantity of seed produced at Site 5 is noteworthy especially in view of the low plant density established in that paddock. Similar seed yields are not uncommon under grazing conditions according to Puckridge and French (1983): however, Carter (1982), who surveyed 35 sites in the cereal-livestock zone of South Australia, found medic seed production to be generally lower than 250 kg/ha and his subsequent measurements have confirmed these trends (Carter 1985, 1986).

The low plant density of medic at Sites 2 and 3 and associated variability in dry matter production also influenced the outcome of the regression analysis (Table 3.4). Highly significant relationships between medic plant density and straw concentration were found at four sites and similarly medic herbage production was heavily dependant on straw concentration at these sites. The values of the coefficients of determination were higher using the exponential model than with the polynomial model and significant correlation was achieved more often with the former model. Equations based on the polynomial model have not been presented.

Finally the results obtained from sampling were considered in a broader perspective in order to assess the impact of the presence of straw walker rows, on a "whole paddock" basis. Taking into consideration the area in each paddock occupied by the walker rows and the reduction in dry matter in the rows, the overall reduction of medic production was derived and found to vary between 12.5 and 22.5 per cent with a mean for six sites of 16.2 per cent (Table 3.5).

TABLE 3.1: Field Survey 1 - mean dry matter yields of medic, green cereal, grass and broadleaf weeds and concentration of straw, at six sites, October 1983.

Site	Position	Dry matter (kg/ha) Medic	Green cereal	Grass	Broadleaf weed	Straw
1	Windrow	88†(4.48)	381 (5.94)	80 (4.38)	18 (2.90)	3876 (8.26)
	Between	1925 (7.56)	69 (4.23)	182 (5.20)	348 (5.85)	722 (6.58)
Signif. of Diff.		***	**	*	***	***
2	Windrow	31 (3.43)	413 (6.02)	41 (3.70)	150 (5.01)	2192 (7.69)
	Between	100 (4.60)	20 (3.01)	26 (3.26)	1343 (7.20)	730 (6.59)
Signif. of Diff.		*	***	ns	***	***
3	Windrow	68 (4.22)	104 (4.64)	775 (6.65)	150 (5.01)	3080 (8.03)
	Between	484 (6.18)	11 (2.38)	236 (5.46)	1469 (7.29)	1529 (7.33)
Signif. of Diff.		***	***	*	***	**
4	Windrow	163 (5.09)	995 (6.90)	12 (2.45)	15 (2.70)	3336 (8.11)
	Between	5782 (8.66)	29 (3.35)	38 (3.64)	393 (5.97)	413 (6.02)
Signif. of Diff.		***	***	**	***	***
5	Windrow	229 (5.43)	694 (6.54)	29 (3.38)	11 (2.38)	3915 (8.27)
	Between	3994 (8.29)	51 (3.92)	100 (4.60)	169 (5.13)	530 (6.27)
Signif. of Diff.		***	**	ns	***	***
6	Windrow	255 (5.54)	2496 (7.82)	0	11 (2.41)	4687 (8.45)
	Between	2496 (7.82)	10 (2.30)	0	75 (4.32)	1227 (7.11)
Signif. of Diff.		***	***		**	***

† Natural value derived from retransformation of log values in parentheses

* P< 0.05; ** P< 0.01; *** P< 0.001; ns, not significant

TABLE 3.2: Field Survey 1 - medic seed reserves and medic plant densities at six sites, October 1983.

Site	Position	Seed reserve (#/m^2)	Plant density (#/m^2)	
1	Walker row	106	10	(2.31)†
	Between	489	169	(5.13)
Signif. of Diff.		*		***
2	Walker row	178	5	(1.69)
	Between	256	16	(2.75)
Signif. of Diff.		ns		*
3	Walker row	50	9	(2.25)
	Between	75	22	(3.11)
Signif. of Diff.		ns		ns
4	Walker row	2785	36	(3.57)
	Between	2618	953	(6.86)
Signif. of Diff.		ns		**
5	Walker row	228	11	(2.35)
	Between	339	99	(4.59)
Signif. of Diff.		ns		***
6	Walker row	4113	65	(4.18)
	Between	2557	620	(6.43)
Signif. of Diff.		*		**

† Natural logarithm; * P< 0.05; ** P< 0.01; *** P<0.001; ns, not significant.

TABLE 3.3: Field Survey 1 - seed production of different species of *Medicago* in walker rows and between rows, December 1983.

Site	Position	*M. truncatula*	*M. minima*	*M. scutellata*	*M. rugosa*	*M. polymorpha*
			(g/m^2)			
1	Walker row	4.68	35.85	-	-	-
	Between	5.88	39.68	-	-	-
Signif. of Diff.		ns	ns			
2	Walker row	-	5.34	13.89	-	-
	Between	-	15.64	2.09	-	-
Signif. of Diff.		ns	**			
3	Walker row	0.52	0.52	-	-	6.92
	Between	9.15	0.98	-	-	10.46
Signif. of Diff.		***	ns			ns
4	Walker row	3.71	48.82	-	-	-
	Between	4.78	54.46	-	-	-
Signif. of Diff.		ns	ns			
5	Walker row	74.45	-	-	12.34	-
	Between	99.95	-	-	3.18	-
Signif. of Diff.		ns			ns	
6	Walker row	6.58	-	-	-	-
	Between	6.06	-	-	-	-
Signif. of Diff.		ns				

** $P<0.01$; ns, not significant

TABLE 3.4: Field Survey 1 - regression coefficients and constants relating (i) medic plant density Y (#/m^2) or (ii) medic herbage production Y (kgDM/ha) to straw concentration X (kg DM/ha), October 1983.

Model $Y=Ae^{BX}$

Site	A	B	$100r^2$	Significance
		(i)		
1	302	-0.00080	81.4	***
2	22	-0.00060	26.7	*
3	37	-0.00040	11.3	ns
4	1255	-0.00096	91.4	***
5	147	-0.00063	86.1	***
6	1388	-0.00064	69.8	***
		(ii)		
1	3769	-0.00091	85.2	***
2	251	-0.00094	52.7	*
3	1781	-0.00098	45.6	*
4	7728	-0.00104	89.3	***
5	4879	-0.00068	76.9	***
6	4503	-0.00057	71.1	***

TABLE 3.5: Field Survey 1 - percentage reduction in medic production in walker rows relative to adjacent areas, proportion of area covered by walker rows and overall percentage loss of medic production.

Site	Stubble type	Medic reduction in walker rows (%)	Area of walker rows in paddock (%)	Total medic reduction (%)
1	Barley	90.1	25	22.5
2	Barley	63.6	25	15.9
3	Wheat	85.9	18	15.5
4	Barley	95.9	13	12.5
5	Barley	89.3	23	20.6
6	Wheat	85.9	12	10.3
				Mean = 16.2

3.2.3 Discussion

The results from this survey confirm the assertions made previously (Andrew 1958; Webber *et al.* 1977; Puckridge and Carter 1980) that high concentrations of cereal residues, especially in straw walker rows, are associated with reduced plant densities of annual medics and concomitant reductions in herbage dry matter production per unit area. This close association between plant density and yield is in agreement with findings of Donald (1951) who found a linear relationship between yield and plant density during the early growth of legume pastures. The limited data collected did not enable any conclusions to be made regarding the fundamental, biological causes for these relationships between straw concentration and medic plant density: however, this was not an objective of this survey but will be examined in subsequent research.

It was evident, both visually and by the data collected, that walker rows were deleterious, but moreover, the curvilinear relationships between straw concentration and medic plant density or dry matter production suggested that the straw concentrations found between walker rows had a harmful impact on dry matter production by medics.

The survey results show that if the cereal residues which pass out of the harvesting machinery were removed from the paddock then a 16 per cent increase in available medic herbage might be expected over the paddock. This amount of feed for livestock is not inconsequential. Further reductions in stubble density by collection and removal of standing stubble would further increase pasture productivity.

The predominant species of medic and mixture of species varied from site to site but regardless of these differences straw residues consistently reduced medic plant densities. However, subtle differences in responses of different medic species and cultivars to high straw concentrations requires more detailed experiments than the research reported here.

Site 3 was one of the two wheat stubbles examined (Site 6 was also wheat) and although there was a weak relationship between straw concentration and medic plant density at this site there was a significant reduction in dry matter production caused by the presence of wheat straw. Only a few wheat stubbles could be considered as potential sites for the survey. Ears of wheat are commonly found at similar heights above the ground

within a single paddock. Consequently harvesting machinery can be adjusted so minimum quantities of straw pass through the machine, therefore concentrations of straw are relatively even over the paddock. Frequently, greater quantities of barley straw are reaped as the cutter bar of the harvester is set closer to the ground because barley plants have a greater tendency than wheat to produce secondary tillers, particularly if late rains occur in the growing season. Furthermore, barley crops are more likely than wheat to lodge in high winds or heavy rain. Greater volumes of straw also pass through the machinery when lodged crops are harvested and higher concentrations of straw are therefore deposited in the walker rows.

3.3 Field Survey 2: Mallala district, Adelaide Plains, 1984

This second survey was intended to examine the effects of varying concentrations of cereal crop residues on developmental phases of annual pasture, including breakdown of hard seed of medics, seedling establishment and survival, together with dry matter production during the growing season. Of special interest were the possible influences of cereal stubble density on the variation in the proportions of hard (impermeable) and soft (permeable) seed reserves of medic in the soil. To investigate these parameters, similar survey techniques were used as in the previous Yorke Peninsula survey together with supplementary studies in the laboratory on seed samples collected during the survey.

The Mallala district was selected for study because of its accessibility to Adelaide and because the environment (soils and climate) and farming practices (integrated cereal and livestock enterprises) are generally representative of a large proportion of the cereal-sheep zone of southern Australia. Detailed descriptions of the climate, soil types and general farming practices for the area are provided by Dahmane (1978), while Webber *et al.* (1976) have provided an overview of the ley farming system in South Australia. The monthly rainfall recorded at the Mallala Post Office during 1984 is shown in Appendix 1.

3.3.1 Materials and Methods

In the Mallala district during early autumn 1984, 13 stubble paddocks were selected which had distinctive straw walker rows left from the preceding grain harvest. Subsequently three of these stubble paddocks were burnt and re-cropped in 1984. Five paddocks were found to have negligible medic emergence after the autumn break (opening rains) so sampling was discontinued. All paddocks were grazed by stock under normal farmer management. Site 3 was the only wheat stubble examined in this survey, all others were barley. One paddock (Site 5) was cut for hay in spring preventing a complete sampling program. Thus four paddocks were sampled *in toto*.

Straw samples were collected in autumn (between April 13 and May 11) at each site, from 10 quadrats (40 x 40 cm) in each of four walker rows and from four adjacent areas between the rows (total 8 strips), to assess straw concentration. In one paddock

germination and emergence of medics had occurred so medic seedlings were counted. Two soil cores, 10.5 cm diameter and 5 cm deep, were taken from alternate quadrats in each strip (i.e. 10 cores/strip) using a Coile soil corer. Each core was transferred with minimal disturbance to a plastic cylindrical tub with a diameter of 11.0 cm. Samples were stored at 19°C for several days then watered to field capacity. After 14 days the emerged medic seedlings were counted and used as an estimate of permeable seed. The remaining seed in each core was extracted from the soil and counted.

In late winter (August), after the break of season and consequent emergence of seedlings, medic plant densities were counted on the quadrat areas in walker rows where the straw had been removed in autumn. The density of medic plants from this count was compared with medic densities in walker rows where straw had not been removed. This comparison was made in order to find whether straw residues reduced seedling emergence *per se*, apart from its effects on seed permeability. A further five quadrats in each of the eight strips were sampled to determine dry matter concentration of straw, medic plant density, medic herbage production and herbage production by grasses and broadleaf weeds.

In mid-October, five samples were taken from each of the eight strips at each site to measure medic plant density and residual straw concentration. *Medicago truncatula* was the predominant legume at all sites except for Site 2 where *M. polymorpha* was the most abundant species of legume.

The relationship between medic plant density or medic dry matter production and straw concentration were initially examined by use of scatter diagrams which indicated that most of them were curvilinear. In order to define these relationships more precisely, six alternative mathematical models were selected on the basis that they are commonly encountered in biological systems (Little and Hills 1978) and regression analysis undertaken using 'Statpak' software and an Apple Macintosh® computer. The equations for the models, together with the coefficients of determination obtained from the analyses, are shown in Tables 3.8 and 3.9. The values of the coefficients were compared to determine the most acceptable model.

3.3.2 Results

The large volume of stubble present was the consequence of a favourable growing season in the previous year which permitted extensive vegetative growth by cereal plants (Table 3.6). Heavy concentrations of cereal residues in walker rows markedly reduced medic plant density at four sites with concomitant reductions in medic dry matter production per unit area. Plates 3.2 and 3.3 show typical plant densities in August for Sites 1, 3, 4 and 5. Plates 3.4 and 3.5 show representative plant densities in October for Sites 1, 2, 3 and 4.

For all sites, emergence counts made on watered soil cores indicated a higher proportion of permeable seed in cores collected from between walker rows compared with those taken from walker rows (Table 3.7). These trends in seedling emergence measured in the laboratory were generally similar to differences in plant density measured later in the field but the seedling emergence from the soil cores (collected from between windrows) was less than the plant densities which established in the field. Watering the soil cores to field capacity may have promoted increased pathogen attack of seedlings, especially when seed was deeply buried and may account for this discrepancy. As well there was more time for seed softening in the field. These results indicate that the concentration of cereal residues on the soil surface over summer and autumn influenced the breakdown of hard-seededness of medic seeds in the soil. Seed numbers in the cores were poorly related to the numbers of seedlings counted in them (Table 3.7), so that the percentage emergence varied widely from site to site (0 to 24).

Within each walker row, straw had been removed from 10 quadrat areas in April. In August, mean density of medic plants in five of these areas i.e. those which did not have soil cores removed in April, was compared with medic density where straw had not been removed. At Sites 1 and 2 the presence of straw between April and August markedly reduced medic density in the walker rows compared with straw-free areas in the walker rows (0 vs 16, 11 vs 32 plants/m^2) (Table 3.6). Emergence of seedlings from soil cores taken from these two sites in April (Table 3.7) is evidence that permeable seed was present beneath the walker rows, therefore the presence of straw between April and August actually inhibited establishment of medic seedlings, possibly by mechanical impedance of

emerging seedlings or by stimulation of pathogen attack during seedling emergence, or a combination of both. At the three remaining sites there were no differences between the two medic plant densities within walker rows but medic density between the rows was always substantially higher. The concentrations of straw at the first two sites were similar to those at the other three sites, so variation in the physical configuration of the straw, particle size or cultivar effects may have been responsible for the different effects of the residues in the walker rows.

The exponential equation was consistently the best fit to the data therefore the regression coefficients and constants derived from the analyses are detailed in Table 3.10. Graphical representation of medic plant density or herbage production over the range of straw concentrations is provided in Figures 3.1, 3.2, 3.3 and 3.4. for the August and October sampling. Medic growth in October, at Site 3, was least affected by straw; 4100 kg/ha straw reduced medic dry matter by half. In contrast, medic growth in the other three paddocks was more severely affected by straw, only 840-1170 kg/ha straw reduced medic dry matter by half. The fit of the curve to Site 3 data was the least satisfactory.

Commonly there was wide variation in medic plant density at low straw concentrations (see Figure 3.1 and 3.3). Removal of young medic plants during grazing by sheep is a likely cause of much of this variation. If grazing had been prevented then plant densities would generally have been higher and the variability less, also the data would have given a closer fit to the exponential model.

3.3.3 Discussion

In this Mallala survey the responses of medic swards to varying concentrations of straw were in close agreement with results from Field Survey 1 on Yorke Peninsula. Observations over the entire growing season indicated that medic plant density was lower under high concentrations of cereal residues early in the season and that subsequent changes in density, during August to October, were minor compared with the initial variation caused by different quantities of straw.

The values of the coefficient "A", determined in the regression analyses (Table 3.10), represent the maximum plant density or dry matter yield of medic at each site when straw

concentration was zero. The value of this coefficient varied from site to site chiefly because of differences in the seed reserves in the soil which imposed limits on plant densities. The values would also have been influenced by environmental factors which affected seed germination, seedling emergence and plant establishment such as seed depth in the soil, incidence of insects and pathogens, grazing pressure, soil texture etc. The coefficient "B" is a rate constant which reflects the sensitivity of medic to changes in straw concentration. For a proportional change in medic density or D.M. yield, eg. 10 per cent, if B is a large value then only a small additional quantity of straw is required to effect the change. As a further illustration, the regression analyses indicated that Sites 1 and 3 had similar D.M. yields of medic, i.e. approximately 670 kg/ha when straw was absent (Table 3.10). To reduce this to 10 per cent i.e. to 67 kg/ha, then ln 10/B = 2.3026/0.000675 = 3410 kg/ha of straw would need to be present at Site 1 and 2.3026/0.000188 = 12,250 kg/ha of straw would be required at Site 3. The value of "B" depends on the physical or chemical characteristics of the straw. It is also influenced by the physical degree of separation between the straw and medic seeds/seedlings/plants. Furthermore, the variation in the intrinsic sensitivity of different medics would affect the value of "B".

Results obtained from the moistened soil cores is strong evidence that the amount of ground cover present over summer and autumn can affect the density of seedlings. This may be attributed to retarded breakdown of hard seed as a consequence of moderated fluctuations in soil temperature below the straw. Various amounts of dry pasture residues have been shown previously to affect changes in seed coat permeability of subterranean clover (Quinlivan 1961; Quinlivan and Millington 1962): however, the relationship was not quantified and the amounts of pasture residues were not stated. Similarly, Burton (1964) obtained evidence that cereal residues could affect changes in the proportion of hard seed at the end of autumn and he found a curvilinear relationship between the amount of straw which covered seed and the amount of permeable seed at the end of autumn. Burton only studied changes in medic seed permeability in one paddock of wheat stubble so he was unable to discuss the possibility of variation in effects between sites. Prior to the studies reported here, there was limited data describing the repercussions of these straw effects on

medic seed in the field. Therefore the additional data from the Mallala survey considerably increases our understanding of pasture growth.

As in the Yorke Peninsula survey, the wheat stubble (Site 3) had less effect than barley stubble on medic plant density and productivity. This may be a consequence of the difference in the particle sizes for each species as wheat was noted to have coarser straw while barley had a larger proportion of residue made up of small particles, derived from awns, other components of the spikelet and macerated pieces of leaf and stem tissue. Barley straw appeared to be more brittle and broke up readily during reaping. These small particles were observed to be more compacted than wheat residues in the walker rows.

It is noteworthy that evidence was found for impairment of seedling emergence which could not be explained solely by effects of straw on hard-seededness (Table 3.6). The two sites where this additional retardation occurred (Sites 1 and 2) had straw concentrations similar to other sites. There were insufficient variables examined to determine the exact nature of the cause of the effect but the magnitude of the reduction in plant density was such that further detailed investigation was warranted. Some possible mechanisms have been examined in experiments described in later sections of this thesis.

TABLE 3.6: Field Survey 2 - medic plant density, medic yield and straw concentration at five sites at Mallala, 1984.

Site	Position	Medic plant density (#/m^2)			Medic yield (kgDM/ha)		Straw concentration (kgDM/ha)		
		Aug(-)†	Aug.	Oct.	Aug.	Oct.	Apr.	Aug.	Oct.
1	Walker row	16	0	1	0	4	8994	8393	5759
	Between		163	127	88	476	1893	1735	944
	Signif. of Diff.		***	***	***	***	***	***	***
2	Walker row	32	11	11	6	85	6808	5897	4151
	Between		48	50	43	995	1630	1449	708
	Signif. of Diff.		***	***	***	***	***	***	***
3	Walker row	45	43	47	28	551	6442	6121	4079
	Between		80	67	67	517	2358	1619	1136
	Signif. of Diff.		*	ns	*	ns	***	***	***
4	Walker row	51	60	52	143	737	5396	2984	3044
	Between		174	151	564	3371	1826	749	685
	Signif. of Diff.		***	***	***	***	***	***	***
5	Walker row	21	23	-	12	-	9174	5686	-
	Between		206	-	26	-	1498	748	-
	Signif. of Diff.		***		***		***	***	

† Straw removed from quadrat area in April.

* $P<0.05$; *** $P<0.001$; ns, not significant

TABLE 3.7: Field Survey 2 - seed numbers in the soil cores collected from beneath walker rows and areas between rows in April/May and emergence of medic seedlings from these cores after 14 days at 19^0C.

Site	Position	Seed number (#/m^2)	Seedling emergence (#/m^2)	(%)
1	Walker row	1009	19.9	1.9
	Between	901	56.9	5.9
2	Walker row	248	22.8	8.4
	Between	147	48.3	24.7
3	Walker row	157	0	0
	Between	147	22.8	13.4
4	Walker row	1260	14.2	1.1
	Between	978	19.9	2.0
5	Walker row	676	22.8	3.3
	Between	428	71.1	14.3

TABLE 3.8: Field Survey 2 - coefficients of determination obtained using six regression models relating medic plant density (#/m^2) to straw concentration (kgDM/ha) at Mallala, 1984.

	I	$Y=A+BX$
	II	$Y=Ae^{BX}$
Model	III	$Y=A+B\log X$
	IV	$Y=AX^B$
	V	$Y=A+BX+CX^2$
	VI	$Y=A+BX+CX^2+DX^3$

Site	***Model***					
	I	II	III	IV	V	VI
			August			
1	0.822	0.937	0.807	0.934	0.841	0.864
2	0.460	0.640	0.469	0.555	0.473	0.473
3	0.107	0.042	0.104	0.031	0.112	0.113
4	0.374	0.451	0.356	0.346	0.393	0.398
5	0.640	0.704	0.618	0.596	0.657	0.665
			October			
1	0.741	0.872	0.783	0.899	0.814	0.814
2	0.686	0.649	0.627	0.541	0.708	0.711
3	0.181	0.248	0.166	0.189	0.181	0.183
4	0.595	0.713	0.562	0.632	0.597	0.605

TABLE 3.9: Field Survey 2 - coefficients of determination obtained using six regression models relating medic production (kgDM/ha) to straw concentration (kgDM/ha) at Mallala, 1984.

Model	I	$Y = A+BX$
	II	$Y=Ae^{BX}$
	III	$Y=A+B\log X$
	IV	$Y=AX^{B}$
	V	$Y=A+BX+CX^{2}$
	VI	$Y=A+BX+CX^{2}+DX^{3}$

Site	*Model*					
	I	II	III	IV	V	VI
			August			
1	0.670	0.881	0.659	0.881	0.685	0.705
2	0.320	0.519	0.324	0.520	0.344	0.347
3	0.094	0.117	0.102	0.115	0.113	0.116
4	0.518	0.584	0.488	0.469	0.549	0.576
5	0.359	0.683	0.365	0.634	0.379	0.380
			October			
1	0.588	0.851	0.635	0.886	0.653	0.655
2	0.613	0.781	0.649	0.683	0.661	0.663
3	0.132	0.120	0.084	0.082	0.136	0.177
4	0.766	0.762	0.761	0.705	0.799	0.817

TABLE 3.10: Field Survey 2 - regression coefficients and constants relating (i) medic plant density Y (#/m^2) or (ii) medic herbage production Y (kgDM/ha) to straw concentration X (kgDM/ha), October 1984.

Model $Y=Ae^{BX}$

Site	A	B	$100r^2$	Significance
		(i)		
1	226	-0.000863	87.2	***
2	70	-0.000539	64.9	***
3	84	-0.000317	24.8	***
4	202	-0.000519	71.3	***
		(ii)		
1	672	-0.000675	85.1	***
2	1472	-0.000833	78.1	***
3	661	-0.000188	12.0	*
4	5134	-0.000722	76.2	***

* $P<0.05$; *** $P<0.001$

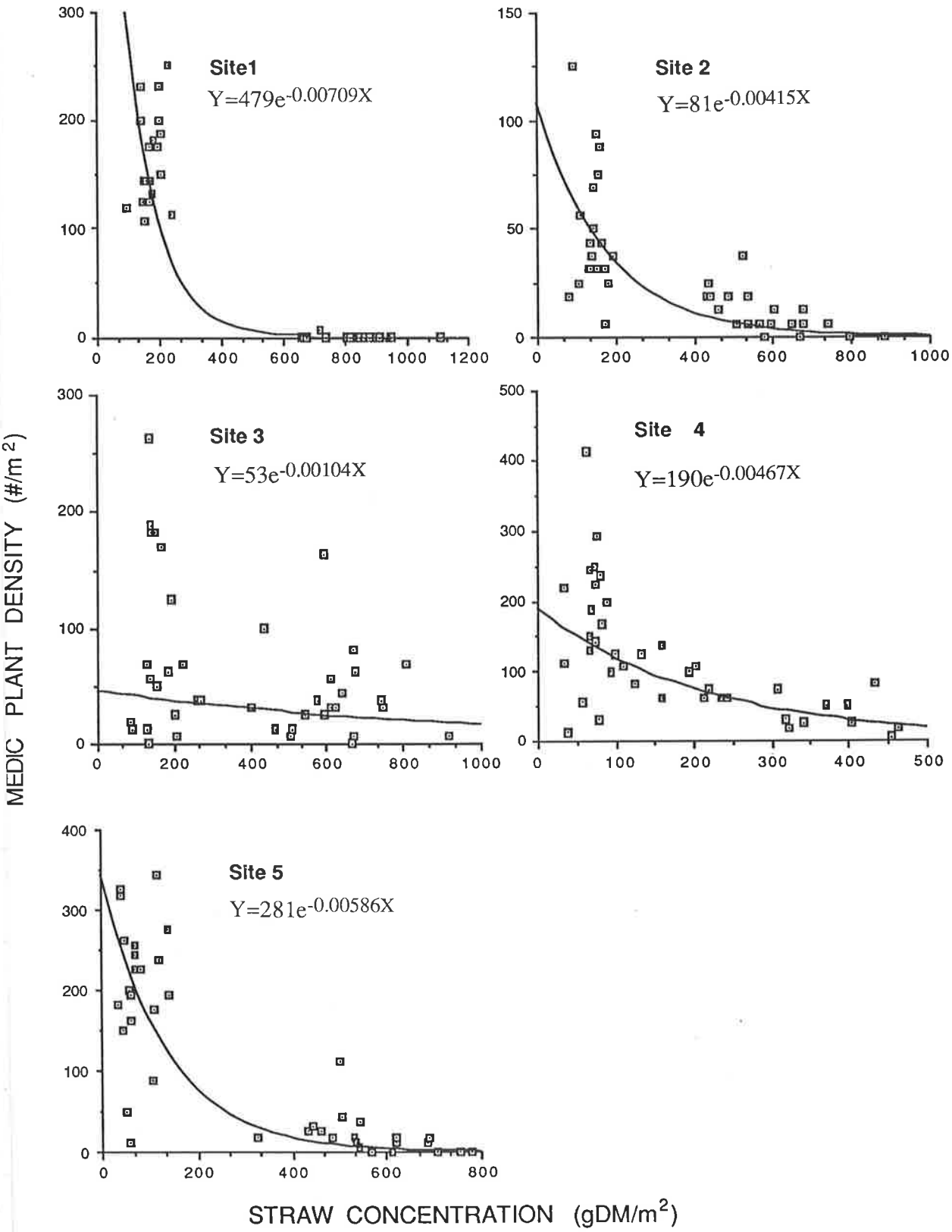


FIGURE 3.1: Field Survey 2 - straw concentrations in five paddocks and the densities of medic plants associated with the straw at Mallala, August 1984. Fitted lines to the data are derived from the exponential model.

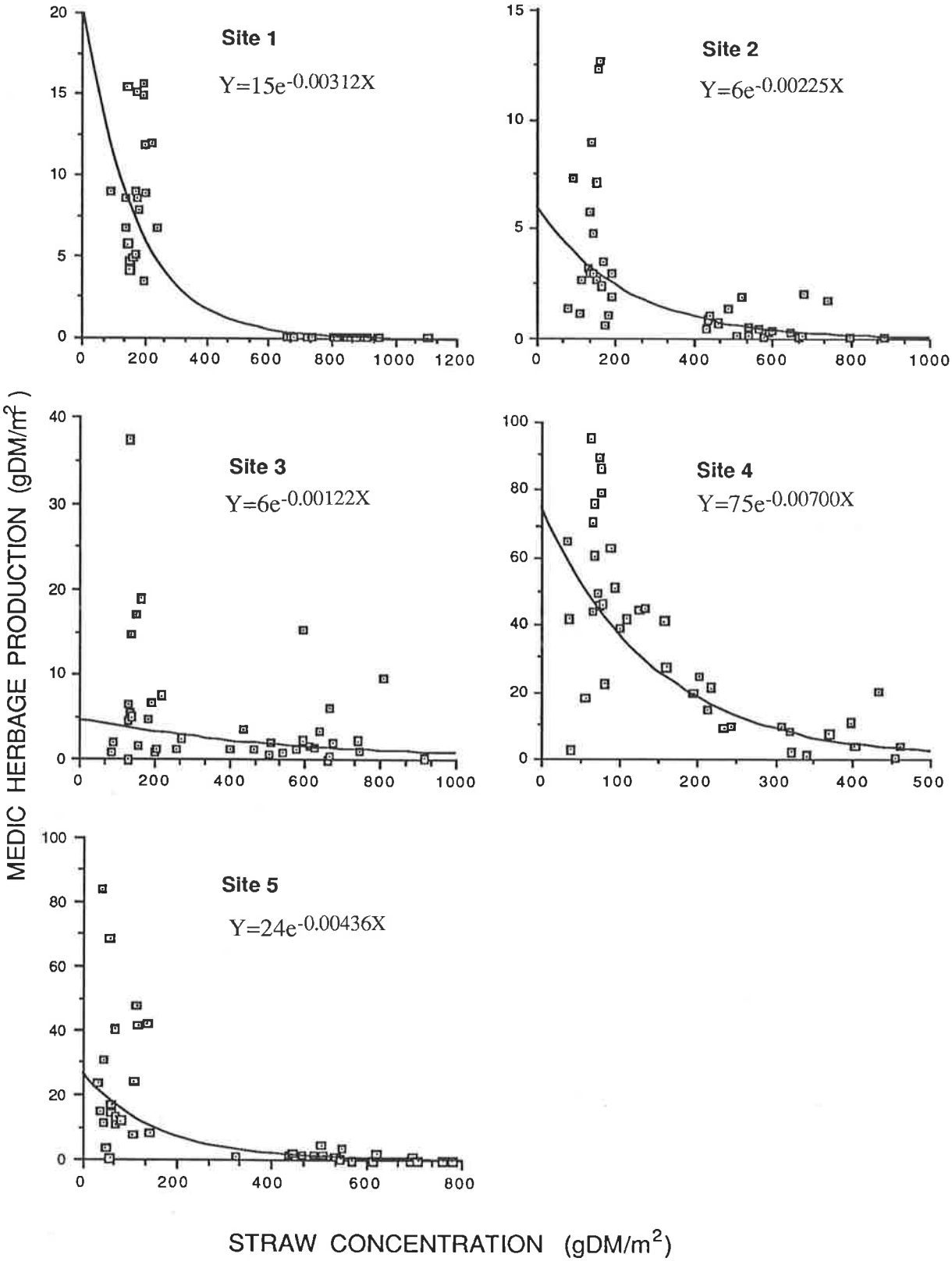


FIGURE 3.2: Field Survey 2 - straw concentrations in five paddocks and the dry matter production of medic associated with the straw at Mallala, August 1984. Fitted lines to the data are derived from the exponential model.

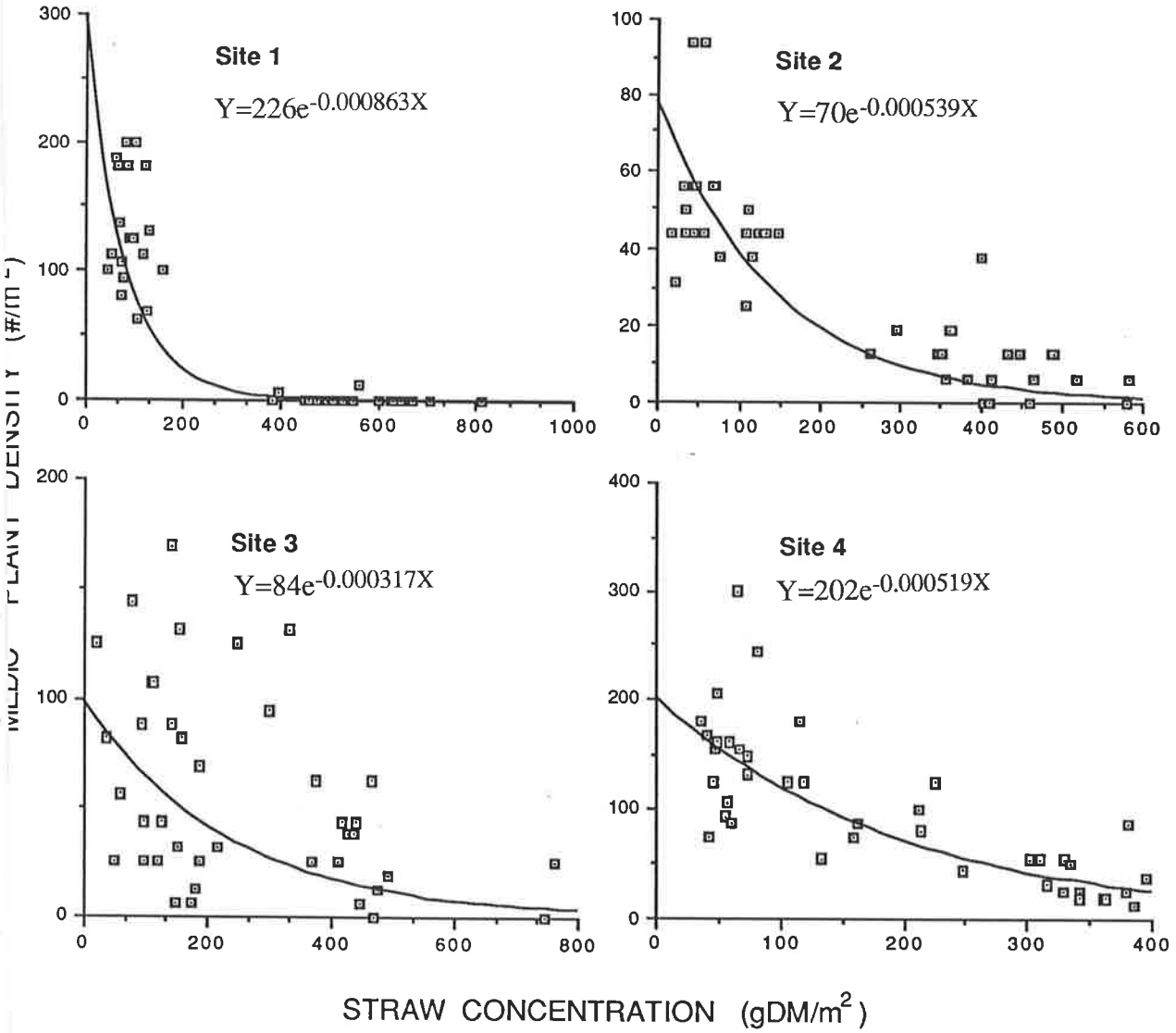


FIGURE 3.3: Field Survey 2 - straw concentrations in four paddocks and the densities of medic plants associated with the straw at Mallala, October 1984. Fitted lines to the data are derived from the exponential model.

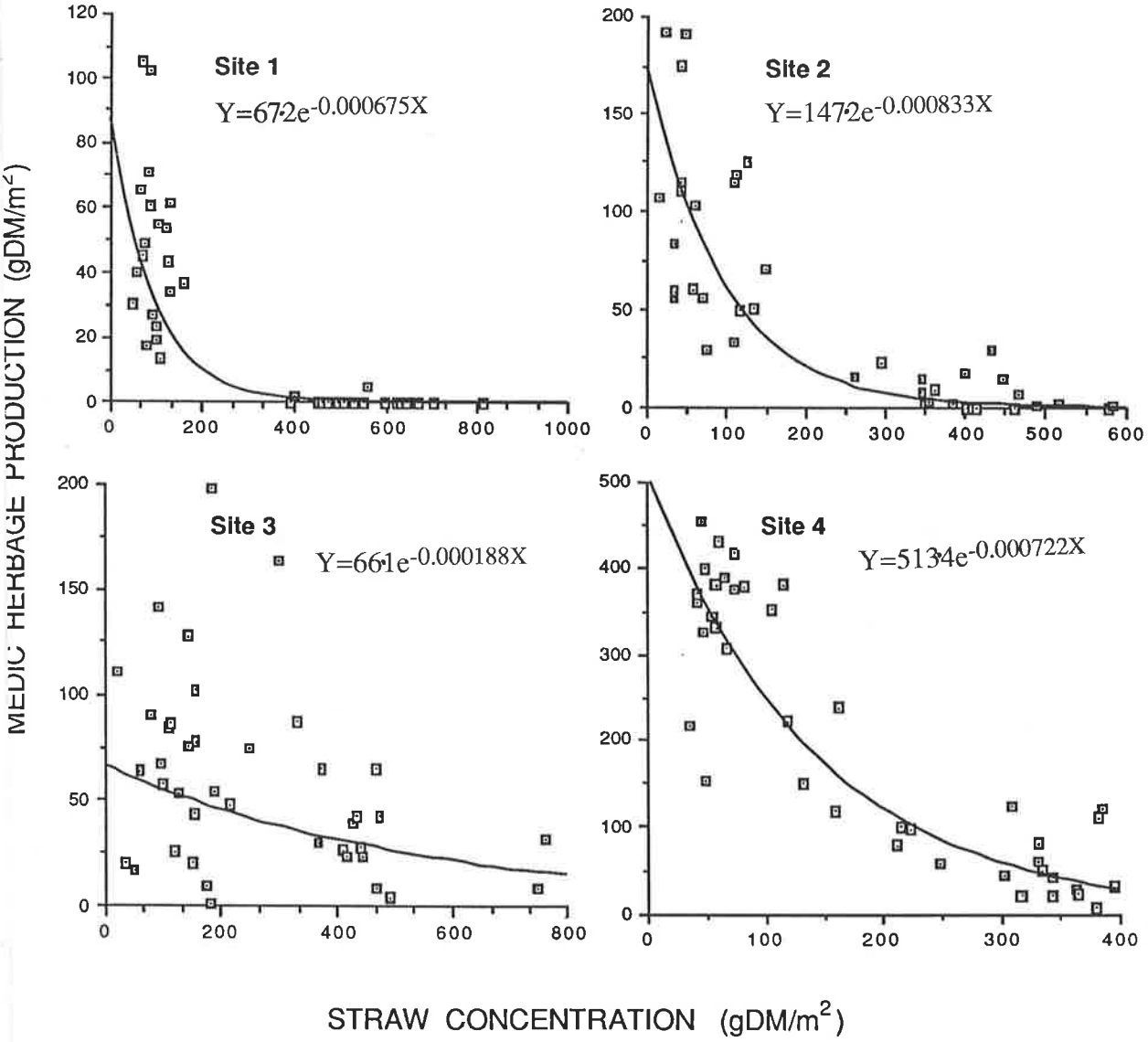


FIGURE 3.4: Field Survey 2 - straw concentrations in four paddocks and the dry matter production of medic associated with the straw at Mallala, October 1984. Fitted lines to the data are derived from the exponential model.

PLATE 3.2: Field Survey 2 - Typical differences in medic plant densities, Sites 1 and 3, at Mallala, August 1984.

A - Site 1, Walker row

B - Site 1, Between walker rows

C - Site 3, Walker row

D - Site 3, Between walker rows

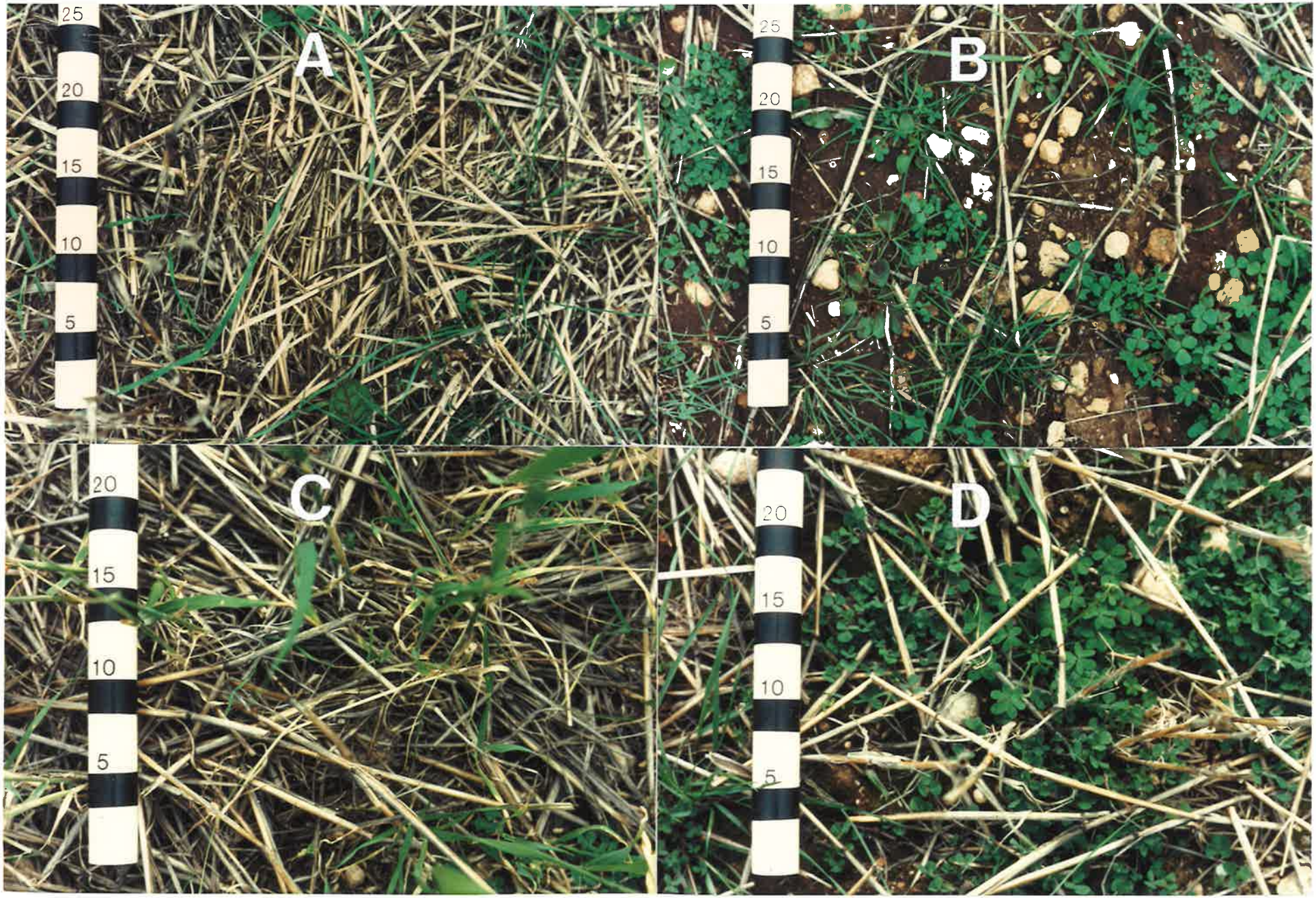

A
B
C
D
25
20
15
10
5

PLATE 3.3: Field Survey 2 - Typical differences in medic plant densities,Sites 4 and 5, at Mallala, August 1984.

A - Site 4, Walker row

B - Site 4, Between walker rows

C - Site 5, Walker row

D - Site 5, Between walker rows

Note the non-legume weed *Oxalis pes-caprae* which is frequently confused with trifoliate legumes.


A
B
C
D

PLATE 3.4: Field Survey 2 - Typical differences in medic plant densities,Sites 1 and 2, at Mallala, October 1984.

A - Site 1, Walker row

B - Site 1, Between walker rows

C - Site 2, Walker row

D - Site 2, Between walker rows

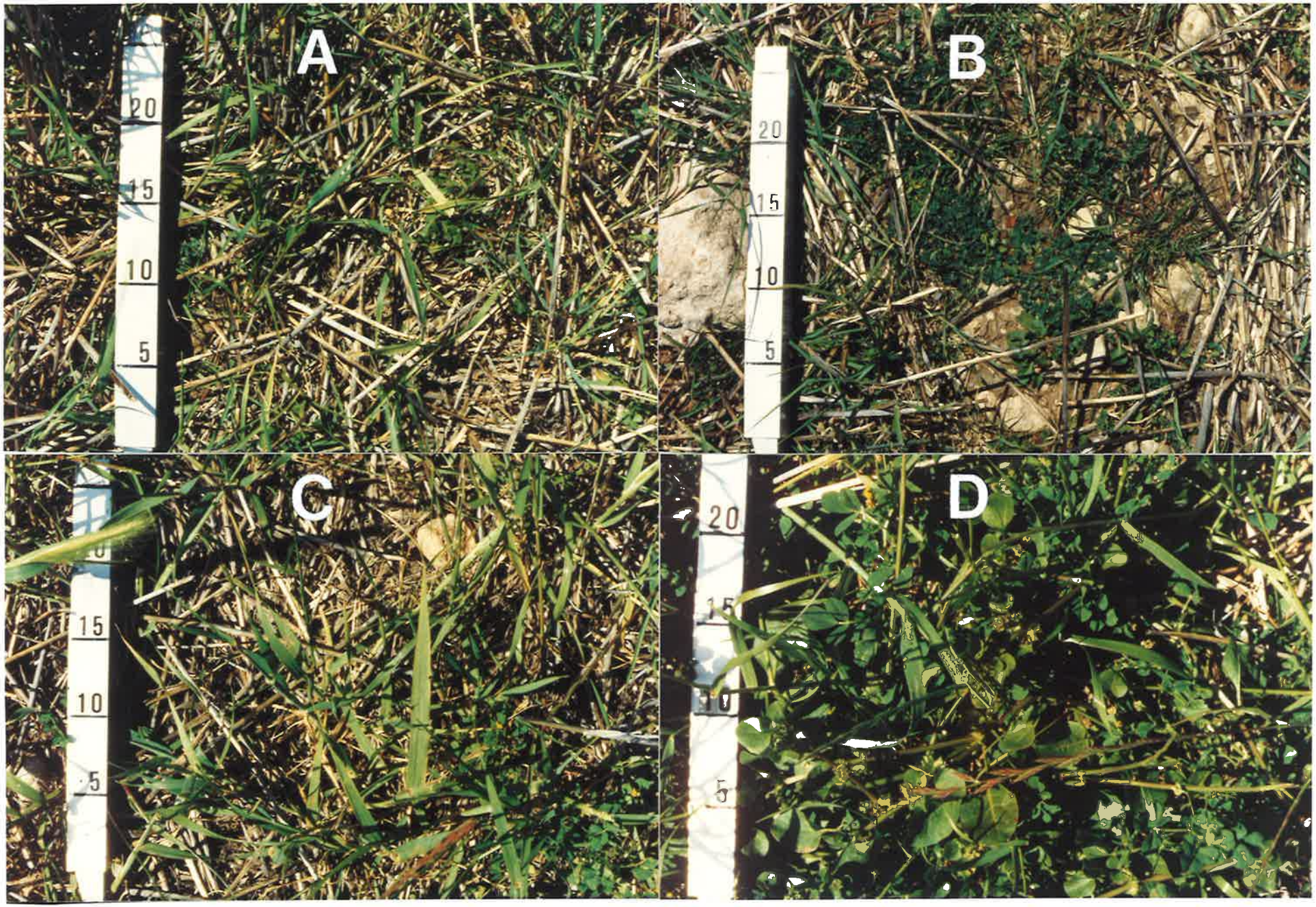

A
20
15
10
5
B
20
15
10
5
C
15
10
5
D
20
15
10
5

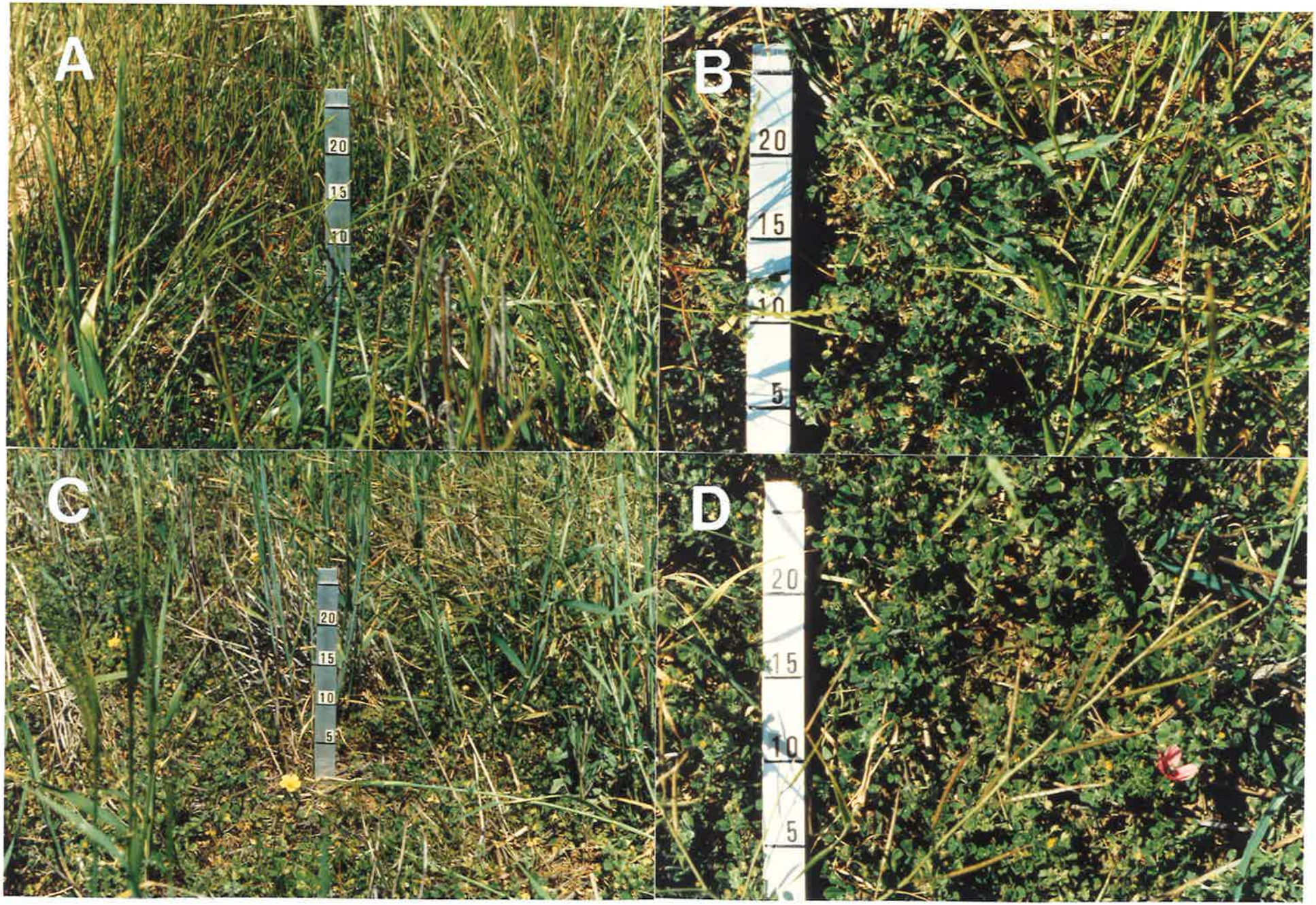
PLATE 3.5: Field Survey 2 - Typical differences in medic plant densities, Sites 3 and 4, at Mallala, October 1984.

A - Site 3, Walker row

B - Site 3, Between walker rows

C - Site 4, Walker row

D - Site 4, Between walker rows


A
20
15
10
B
20
15
10
5
C
20
15
10
5
D
20
15
10
5

3.4 Field Survey 3: Mallala district, Adelaide Plains, 1985

The Mallala field survey was repeated during the 1985 growing season as it was considered necessary to substantiate the conclusions drawn from the previous surveys and to further examine the variation between sites. The sites were again located in the Mallala district so that direct comparisons could be made between results obtained from the 1984 and 1985 surveys.

A new objective was to examine the hard-seededness of medic seed reserves left after the initial seed germination and emergence in the field. Taylor (1981), working with subterranean clover seed has shown that seed which remains hard after exposure to high temperatures may subsequently develop a different pattern of breakdown compared with seeds previously exposed to lower temperatures. Medic seed has not been examined for similar effects, thus seeds collected from beneath walker rows or between rows where different amounts of cereal residue covered the soil so moderating the temperatures to which the seed was exposed over summer and autumn, could provide a useful comparison.

The intrinsic patterns of change in hard-seededness of annual medic seed, when exposed to regular cycles of alternating temperatures, has been examined by Quinlivan (1961) who investigated a single genotype of *M. truncatula*. More recently, Bolland (1986) measured the pattern of softening of hard seeds of a range of annual medic species under laboratory conditions. The investigation reported here differs from these other assessments because the seed was retrieved from soil in paddocks and therefore was representative of the actual seed reserve. In contrast Quinlivan and Bolland studied freshly-grown seed. The pattern of breakdown of hard seed from Mallala was examined by inducing changes in seed coat permeability through the imposition of diurnally fluctuating temperatures in a laboratory incubator.

3.4.1 Materials and Methods

Agronomic measurements in the field

A survey of selected paddocks in the Mallala district was commenced in mid-May 1985. Five paddocks were chosen which had distinctive straw walker rows present (all

barley). These areas were subsequently grazed by livestock under farmer management. Rainfall for each month was recorded at Mallala Post Office and is tabulated in Appendix 1.

After the first general emergence of annual medics in the field during early May, samples were collected at four sites, from five quadrats (40 x 40 cm) in each of three walker rows and three adjacent areas, except at Site 3 where three quadrats were used as the density of medic plants was higher and less variable. The number of medic plants in each quadrat was counted and the plants cut at ground level using knives for dry matter determination; all cereal straw within the quadrat was also collected. Five soil cores 10.5 cm diameter and 5 cm deep were taken from each quadrat area using a Coile sampler and bulked. Plant material was sorted by hand then dried in a forced-draught dehydrator at 80^{o}C for 24 hours before being weighed.

The soil cores were transferred to a 0.7 mm square-mesh sieve and gently agitated in water. After most of the clay and silt had passed through the sieve the vegetable matter and gravel remaining in the sieve was washed into a large plastic beaker partially filled with water. This trapped small stones and gravel at the bottom whilst organic matter was carefully strained from the water. The organic matter and the gravel were then dried at ambient temperature, in a forced-draught dehydrator, for 12 hours. Medic pods were separated from the other vegetable debris and counted and weighed. Any medic seeds mixed with the gravel were picked out by hand and counted. Medic pods were manually threshed by gently rubbing them between two sheets of corrugated rubber mounted on blocks of wood after which seed was separated from the pod debris by sieving and aspiration. Seeds were then counted and weighed.

These procedures for sampling and processing were repeated for four sites in mid-September avoiding quadrat areas previously sampled. At Site 5 no medic plants survived through to September, presumably because of insect attack, so sampling was discontinued.

Medic pod and seed production during the growing season were assessed on December 5 when pod samples were collected from the four paddocks. The samples were gathered from the soil surface within 288 mm diameter steel rings, five samples from each of three straw walker rows and adjacent strips. Dried herbage and soil was separated from

the pods by sieving and hand sorting then the pods were weighed and counted. Seeds were threshed from the pods, counted and weighed. Student's *t* -test was used to compare mean values for densities within walker rows and between walker rows.

Changes in hard-seededness in the laboratory

To examine the pattern of change in seed-coat permeability, seed samples collected in May were transferred into petri dishes with two Whatman® No1 qualitative filter papers moistened with 5 ml of deionized water and then stored at 20°C for 2 days. Germinated seeds were removed after counting and the initial germination percentages calculated.

Quinlivan (1961) found no difference in the rate of seed softening when seed of *M. truncatula* was exposed to either 60/15 or 45/15°C. Alternating temperatures of 60/15°C have commonly been used by other researchers (Collins 1978, 1981; Gladstones 1967; Quinlivan 1965a, 1968a; Taylor 1981, 1984; Taylor and Palmer 1979) as a method of causing hard-seededness to diminish over an extended period but 50/15°C was imposed here as this better represents local soil temperatures experienced over summer. In the current experiment, soil temperatures over summer and autumn were simulated by placing the seed samples in an incubator which maintained a temperature of 50°C for 7 hours and 15°C for 15 hours every day, with transitional temperatures between these intervals. After 24 hours of alternating temperatures the seeds were returned to 20°C in a different incubator and moistened again. After 2 days the germination was measured and the percentage calculated based on the number of hard seeds remaining after the initial germination above and all cumulative germination percentages are based on this number of seeds. The samples were then stored at alternating temperatures for 13 days (14 days cumulative) then germinated by the same processes, this was then followed by additional storage for 14 days (28 cumulative) at alternating temperatures. A pattern was then maintained whereby the seeds were held at alternating temperatures for periods of 28 days followed by germination at 20°C for two days. Results from 17 cycles have now been obtained. Cumulative germinations of seed from each site were compared by Student's *t* -test.

3.4.2 Results

Agronomic measurements from the field

The gross effects of straw walker rows can be assessed from data in Table 3.11 where mean values for straw concentration, medic plant density and medic dry matter yield are presented. The medic species found at Site 5 was almost entirely *M. polymorpha* while *M. truncatula* dominated at all other sites. Large differences in medic herbage production are obvious when mean values for walker rows are compared with means from between rows. The poor productivity within the areas of highest straw concentration was a consequence of reduced medic plant density. In the interval between the two times of sampling there was complete elimination of medics at Site 5, furthermore, the quantities of herbage measured at Site 3 in September were comparatively low in view of the plant density. Most likely this poor productivity at Site 3 was caused by heavy infestation of aphids as large populations of blue-green aphids (*Acyrthosiphon kondoi*) and spotted alfalfa aphids (*Therioaphis trifolii*) were observed at this location during sampling in May. It is possible that insect attack at Site 5 was responsible for the reduction in plant numbers but no infestation was observed. Alternatively, heavy grazing pressure may have been responsible for the reductions in medic plant density.

Regardless of these stresses which occurred through the growing season, pastures at Sites 2 and 3 produced large quantities of seed, as determined in December (Table 3.12). At these two sites there was significantly more seed produced between the straw walker rows than in the rows, but at Sites 1 and 2, lateral growth of medic extended over the walker rows from plants located between rows resulting in no difference in the spatial distribution of new seed production. The large decline in straw concentration (approx. 70 per cent) and low dry matter production by medic over the growing season were indicative of heavy grazing pressure which is consistent with the low medic seed production. Similarly, large changes in the concentrations of cereal straw residue at Site 5 (76 per cent reduction) suggests a heavy grazing pressure which may have contributed to the demise of the medic at this site.

Comparisons of coefficients of determination obtained from the six models

indicated that Model II (exponential) was little better than the others (Tables 3.13 and 3.14), in particular there was little distinction between II and VI (exponential and polynomial respectively): however, Model II remains the preferred equation. As less quadrats were sampled at each site in this survey than the number used in Survey 2 there was less precision in the relationships obtained and consequently the values for coefficients of determination were lower. The relationships between medic plant density or medic dry matter production and straw concentration (Table 3.15) were all highly significant in September as were the equations derived from Model II relating medic plant density in May to straw concentration (not presented). These data have been plotted in Figures 3.5, 3.6 and 3.7.

Changes in hard-seededness in the laboratory

For all five sites there was a higher germination percentage of seed collected from beneath walker rows compared with seed sampled from between rows (Figures 3.8 and 3.9). These differences were manifested early during the assessment (Figure 3.8) and little further divergence of the lines occurred beyond Day 56. For Sites 1, 2 and 4 there was a statistically significant difference ($P<0.05$) in the germinations of the two seed groups, 28 days after initiating temperature treatments in the laboratory. The disparity was the least at Site 3 and not statistically significant. A significant difference ($P<0.05$) was detected between the groups from Site 5, 56 days after commencement.

The rate of change of seed coat permeability was almost linear for 300 days or so but after this time germination of seeds from Sites 1, 4 and 5 showed a reduced tendency to become permeable (Figure 3.9). The pattern of seed germination at Site 2 deviated from the general trend exhibited by the other sites as the rate of seed softening was slower and no change in rate has occurred 500 days after initial exposure to alternating temperatures.

3.4.3 Discussion

This 1985 survey confirmed the findings of the previous two surveys i.e. that medic plant density and yield in walker rows were markedly retarded by high concentrations of cereal straw residues.

Larger quantities of seed reserves were found in 1985 than in Survey 2 in 1984, consequently the densities of established medic plants were higher in 1985 than 1984. However, by spring 1985 the range in medic herbage production between sites was similar to that in 1984.

The data regarding changes in hard-seededness can be compared with the results obtain by Quinlivan (1961) and by Bolland (1986). The samples of seed from Mallala (*M. truncatula* from most sites) had a linear pattern of seed softening at least for the initial 300 days whereas Quinlivan (1961) found that the rate of breakdown of hard-seededness of *M. truncatula* declined substantially after 90 days of diurnally-alternating temperatures (15/60°C). Although tabular data indicated considerable differences between species, the graphs provided by Bolland (1986) depicting changes in hard-seededness were each derived from means of five species of *Medicago*. Therefore, comparisons of his graphs with patterns determined from seed from Mallala must be made with caution. His graphs show slow changes in the proportion of soft seed over the first 70-100 days followed by a long period when a more rapid, constant rate persisted, then sometime before day 300 there was a decline in the rate of seed softening. The germination results from the survey reported here did not show the same early inhibition of hard-seededness breakdown but at three sites there were reductions in the rate of breakdown commencing close to day 300, similar to the pattern described by Bolland. Furthermore, Bolland found that seed of *Medicago polymorpha* cvv. Serena and Circle Valley had only 6 and 7 per cent soft seed respectively, 160 days after alternating temperatures commenced, very much lower than his *M. truncatula* cultivars (28 and 41 per cent). In contrast, the pattern of seed softening of *M. polymorpha* at Site 5 in this Mallala survey was similar to the responses of *M. truncatula* seed from the other four sites.

The higher germination of seed collected from walker row areas compared with seed from between the rows early in the laboratory assessment, concur with the

conclusions of Taylor (1981, 1984). He found that, after retrieving residual hard seed of eight cultivars of subterranean clover which had been buried in the soil at four depths either for 1, 2 or 3 years, the highest germination in the laboratory occurred in seed which had been buried deep and for the longest duration. He argued that seed near the surface had been stimulated sufficiently during the time of burial to induce softening. With increasing depth in the soil, the seed received insufficient thermal stimulus to break hard-seededness. However, the temperature regimes were sufficient to precondition the seed so that when it was retrieved from the field then exposed to alternating temperatures in the laboratory, the seed had a greater tendency to germinate. The magnitude of the difference in germination found in Survey 3 was only about 2 to 10 per cent (Fig 3.8).

The instances where 10 to 25 per cent of the original seed remained hard and showed little tendency to soften in response to alternating temperatures after 300 days, may be of ecological significance. Having this extremely hard-seeded population of seed would be advantageous for the long term persistence of the genotype at any particular location.These seeds were very resistant to effects of diurnal temperature fluctuations in the laboratory although other mechanisms for breakdown of hard-seededness may be effective in the field, such as mechanical scarification during cultivation or chemical effects within the gut of ruminants.

Seed reserves in paddocks have quite different backgrounds compared with freshly- grown seed. The former may consist of seed produced over many seasons during which seasonal conditions may vary, affecting the degree of seed permeability. This seed may be preconditioned by a wide range of temperatures while the seed remains hard. It is important therefore to have some knowledge of patterns of breakdown of hard-seededness in soil seed reserves as these patterns appear different to that reported for fresh seed by Quinlivan (1961) and Bolland (1986). There is a paucity of data describing the potential germination of seed on farms and the effects of local environmental variables on the potential and actual germination levels of seed *in situ*.

TABLE 3.11: Field Survey 3 - medic plant density, medic yield and straw concentration at five sites at Mallala during 1985.

Site	Position	Medic plant density (#/m^2)		Medic yield (kgDM/ha)	Straw concentration (kgDM/ha)	
		May	September	September	May	September
1	Walker row	234	361	549	11170	3168
	Between	593	673	971	7110	2190
Signif. of Diff.		**	**	**	**	**
2	Walker row	100	85	611	5150	3030
	Between	154	196	1410	1800	763
Signif. of Diff.		**	**	**	**	**
3	Walker row	413	291	135	4390	2350
	Between	719	776	756	2310	1210
Signif. of Diff.		**	**	**	**	*
4	Walker row	125	94	660	5540	2460
	Between	266	314	2730	2870	1110
Signif. of Diff.		*	**	**	**	**
5	Walker row	107	0	0	6890	2840
	Between	667	0	0	3440	840
Signif. of Diff.		**			**	**

* P<0.05; ** P<0.01

TABLE 3.12: Field Survey 3 - medic seed reserves in the soil during the growing season and seed production at Mallala in 1985.

Site	Position	Medic seed reserve (0-5 cm soil) May		September		Medic seed production December	
		(#/m^2)	(kg/ha)	(#/m^2)	(kg/ha)	(#/m^2)	(kg/ha)
1	Walker row	3757	113	3168	95	797	24
	Between	3701	112	3602	108	769	22
Signif. of Diff.						ns	
2	Walker row	1623	45	1224	36	4750	120
	Between	1853	51	1486	43	6680	175
Signif. of Diff.						*	
3	Walker row	8213	247	8399	256	9300	296
	Between	9661	292	8264	248	14820	474
Signif. of Diff.						**	
4	Walker row	1377	34	1218	30.0	5680	134
	Between	1439	39	1182	30.3	3900	80
Signif. of Diff.						ns	
5	Walker row	6318	164	-†	-	-	-
	Between	8924	220	-	-	-	-

* P<0.05; ** P<0.01; ns, not significant.
† zero medic plant density in September and seed reserve was not measured at this time.

TABLE 3.13: Field Survey 3 - coefficients of determination using six regression models, relating medic plant density (plants/m^2) to straw concentration (kgDM/ha) at Mallala, 1985.

	I	$Y=A+BX$
	II	$Y=Ae^{BX}$
Model	III	$Y=A+BlogX$
	IV	$Y=AX^B$
	V	$Y=A+BX+CX^2$
	VI	$Y=A+BX+CX^2+DX^3$

Site	***Model***					
	I	II	III	IV	V	VI
				May		
1	0.174	0.270	0.154	0.225	0.175	0.199
2	0.340	0.321	0.441	0.408	0.506	0.681
3	0.609	0.714	0.546	0.615	0.616	0.733
4	0.459	0.473	0.507	0.407	0.490	0.619
5	0.454	0.632	0.438	0.583	0.455	0.468
				September		
1	0.344	0.384	0.258	0.301	0.348	0.423
2	0.478	0.554	0.524	0.603	0.560	0.562
3	0.223	0.374	0.149	0.280	0.226	0.285
4	0.442	0.452	0.449	0.427	0.471	0.479

TABLE 3.14: Field Survey 3 - coefficients of determination using six regression models, relating medic dry matter production (kgDM/ha) to straw concentration (kgDM/ha) at Mallala, September 1985.

	I	$Y=A+BX$
	II	$Y=Ae^{BX}$
Model	III	$Y=A+BlogX$
	IV	$Y=AX^{B}$
	V	$Y=A+BX+CX^{2}$
	VI	$Y=A+BX+CX^{2}+DX^{3}$

Site	*Model*					
	I	II	III	IV	V	VI
1	0.304	0.363	0.206	0.243	0.306	0.376
2	0.491	0.537	0.449	0.486	0.506	0.606
3	0.333	0.388	0.606	0.423	0.543	0.633
4	0.455	0.452	0.391	0.387	0.455	0.514

TABLE 3.15: Field Survey 3 - regression coefficients and constants relating (i) medic plant density Y (#/m^2) or (ii) medic production Y (kgDM/ha) to straw concentration X (kgDM/ha), September 1985.

$$Model \quad Y=Ae^{BX}$$

Site	A	B	$100r^2$	Significance
		(i)		
1	833	-0.000165	38.4	***
2	221	-0.000317	55.4	***
3	869	-0.000463	37.4	**
4	436	-0.000704	45.2	**
		(ii)		
1	1219	-0.000164	36.3	***
2	1642	-0.000344	53.7	***
3	680	-0.000642	38.8	**
4	4934	-0.001000	45.2	**

** P<0.01; *** P<0.001

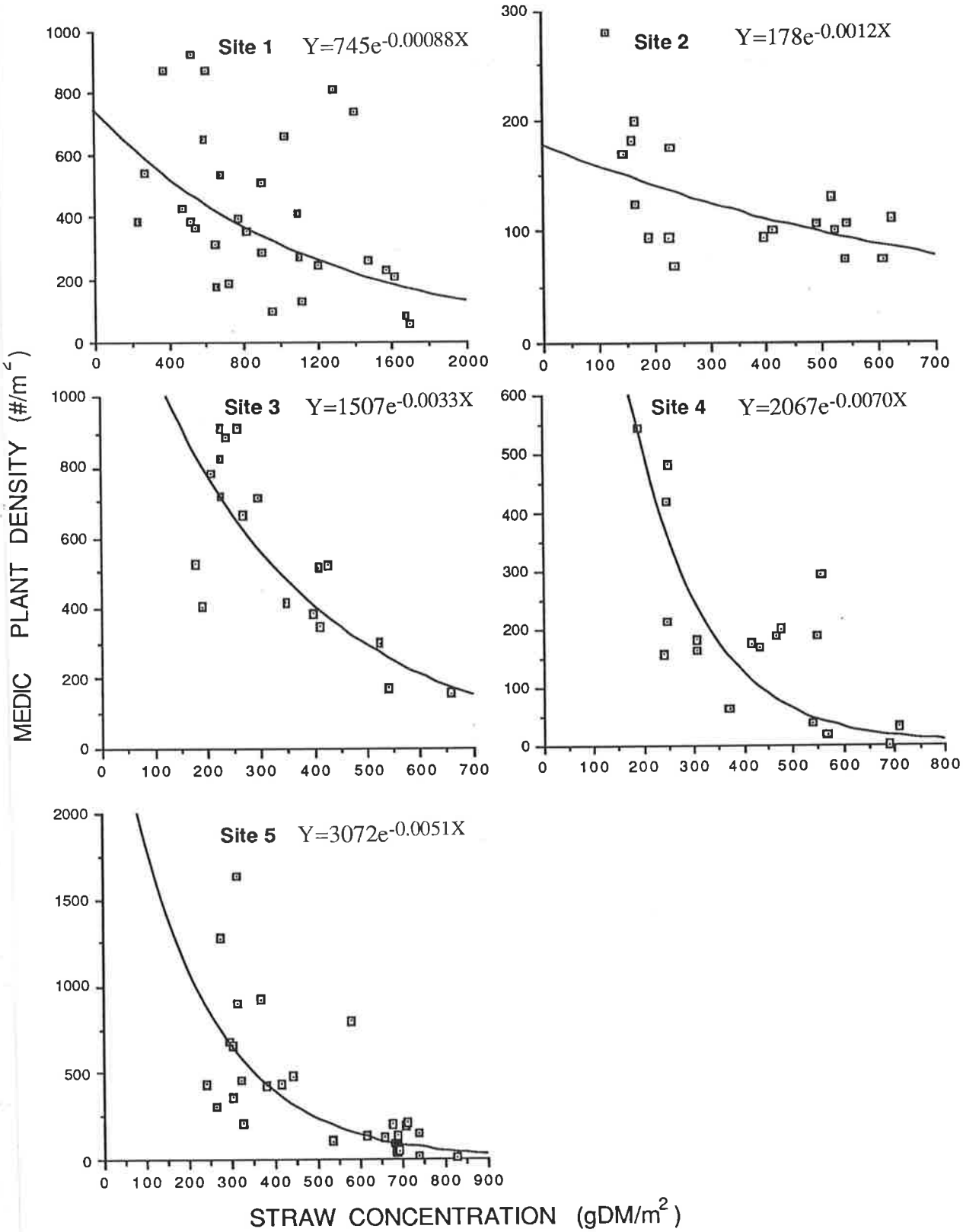


FIGURE 3.5: Field Survey 3 - straw concentrations in five paddocks at Mallala and the densities of medic plants associated with the straw, May 1985. Fitted lines to the data are derived from the exponential model.

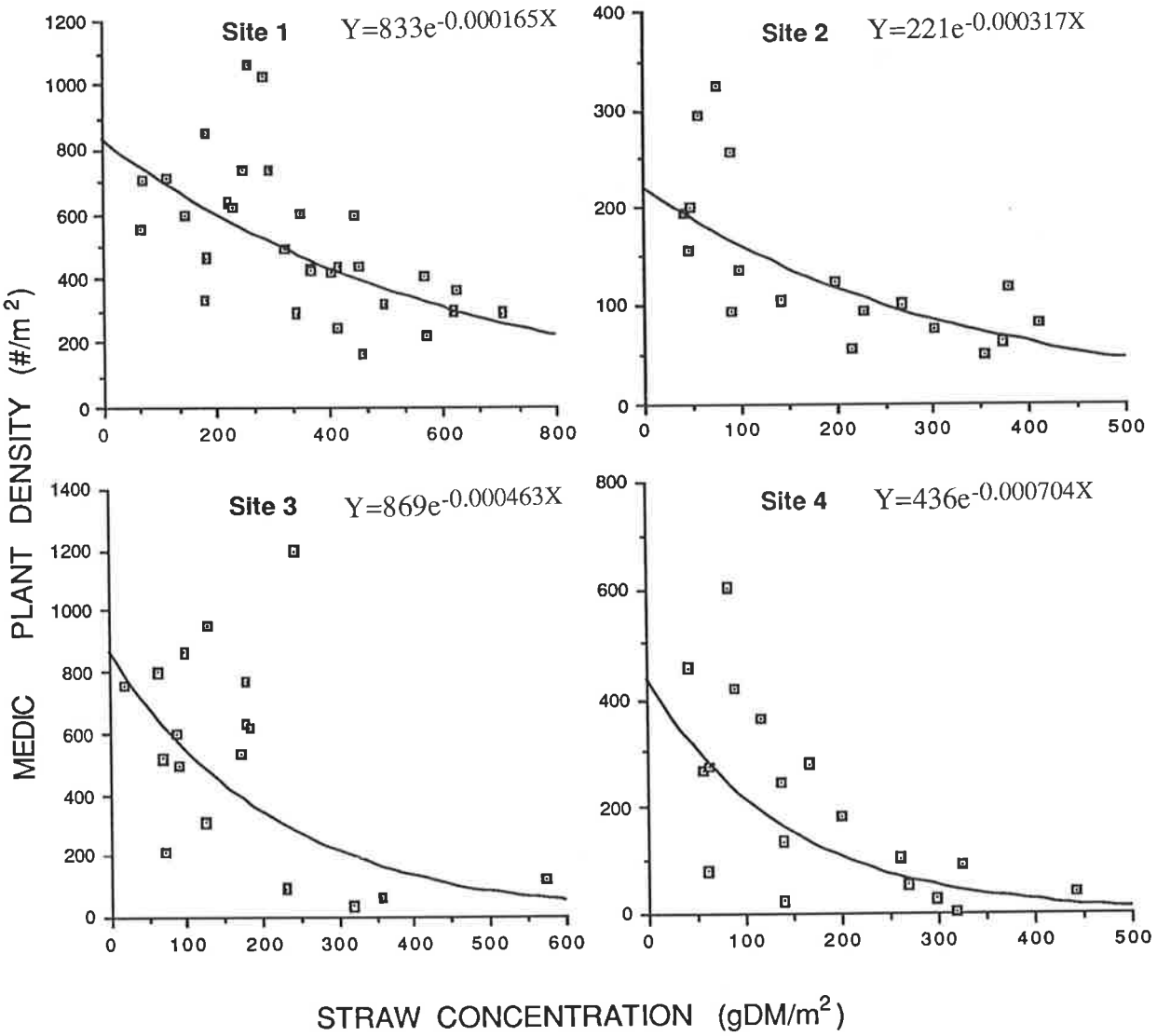


FIGURE 3.6: Field Survey 3 - straw concentrations in four paddocks at Mallala and the densities of medic plants associated with the straw, September 1985. Fitted lines to the data are derived from the exponential model.

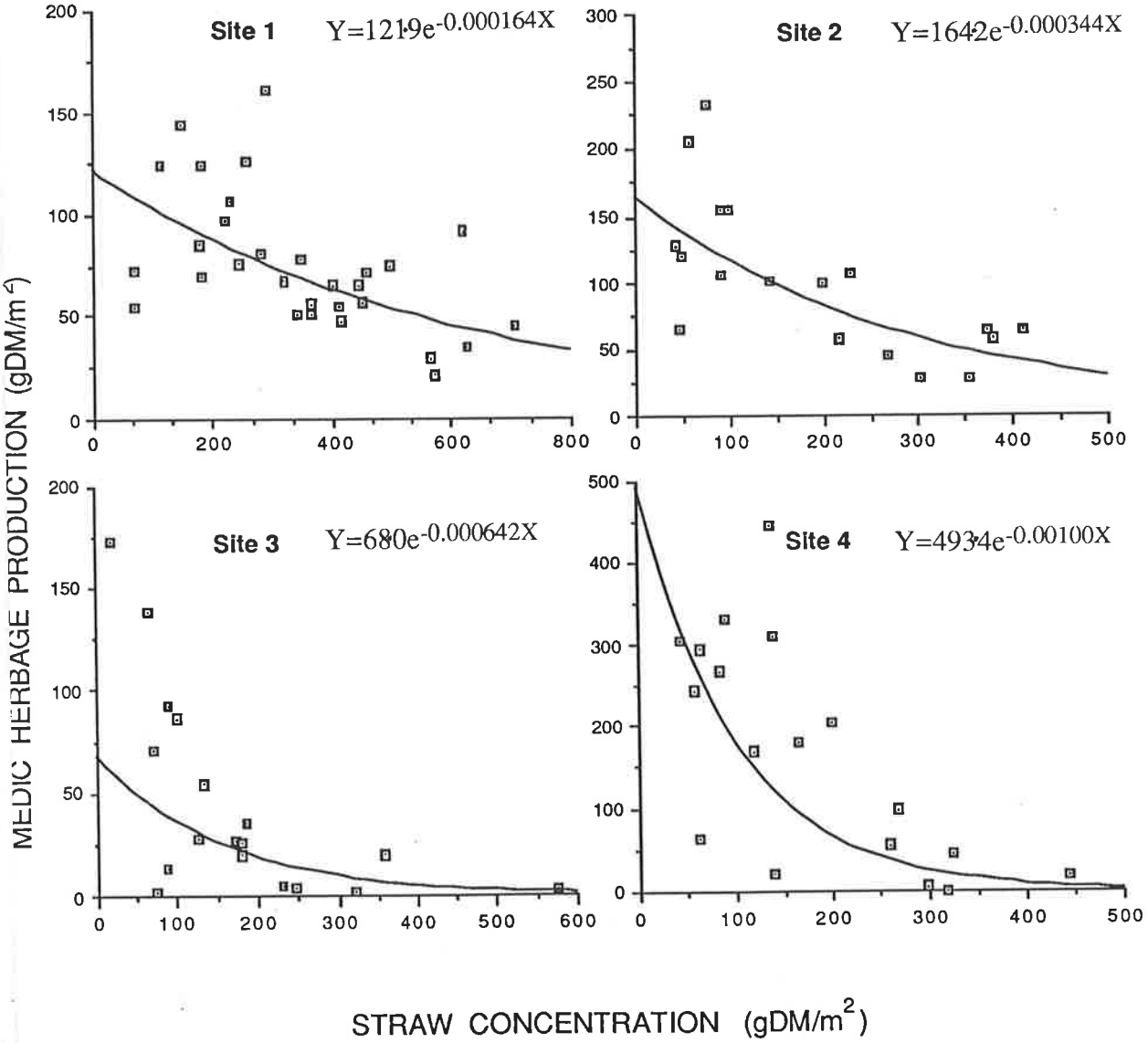


FIGURE 3.7: Field Survey 3 - straw concentrations in four paddocks at Mallala and the dry matter production of medic associated with the straw, September 1985. Fitted lines to the data are derived from the exponential model.

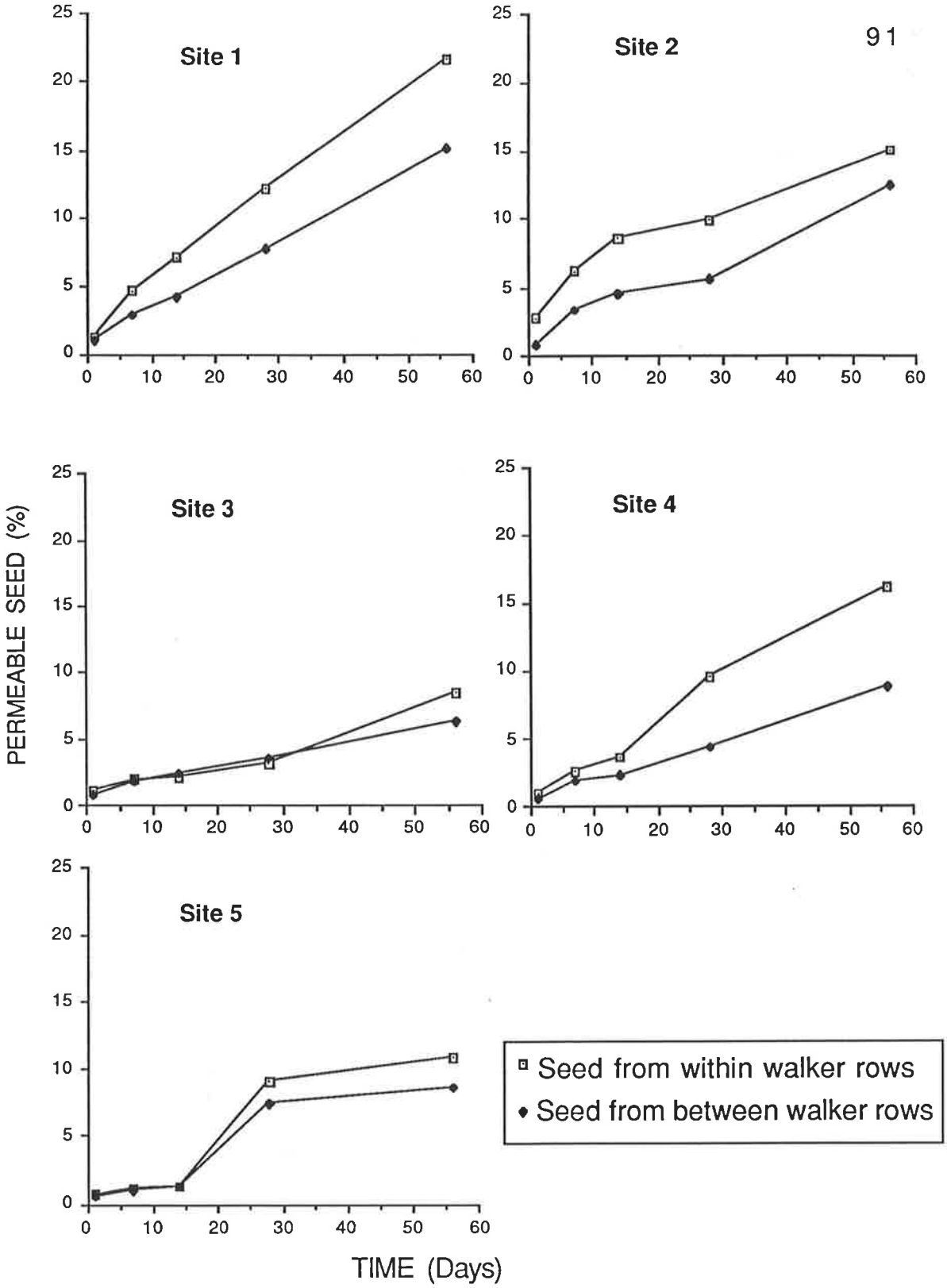


FIGURE 3.8: Field Survey 3 - cumulative germination of medic seed in the laboratory over 60 days. Seed was exposed to diurnally-fluctuating temperatures of 50/15°C except for 48-hour periods when germination percentages were assessed at 20°C.

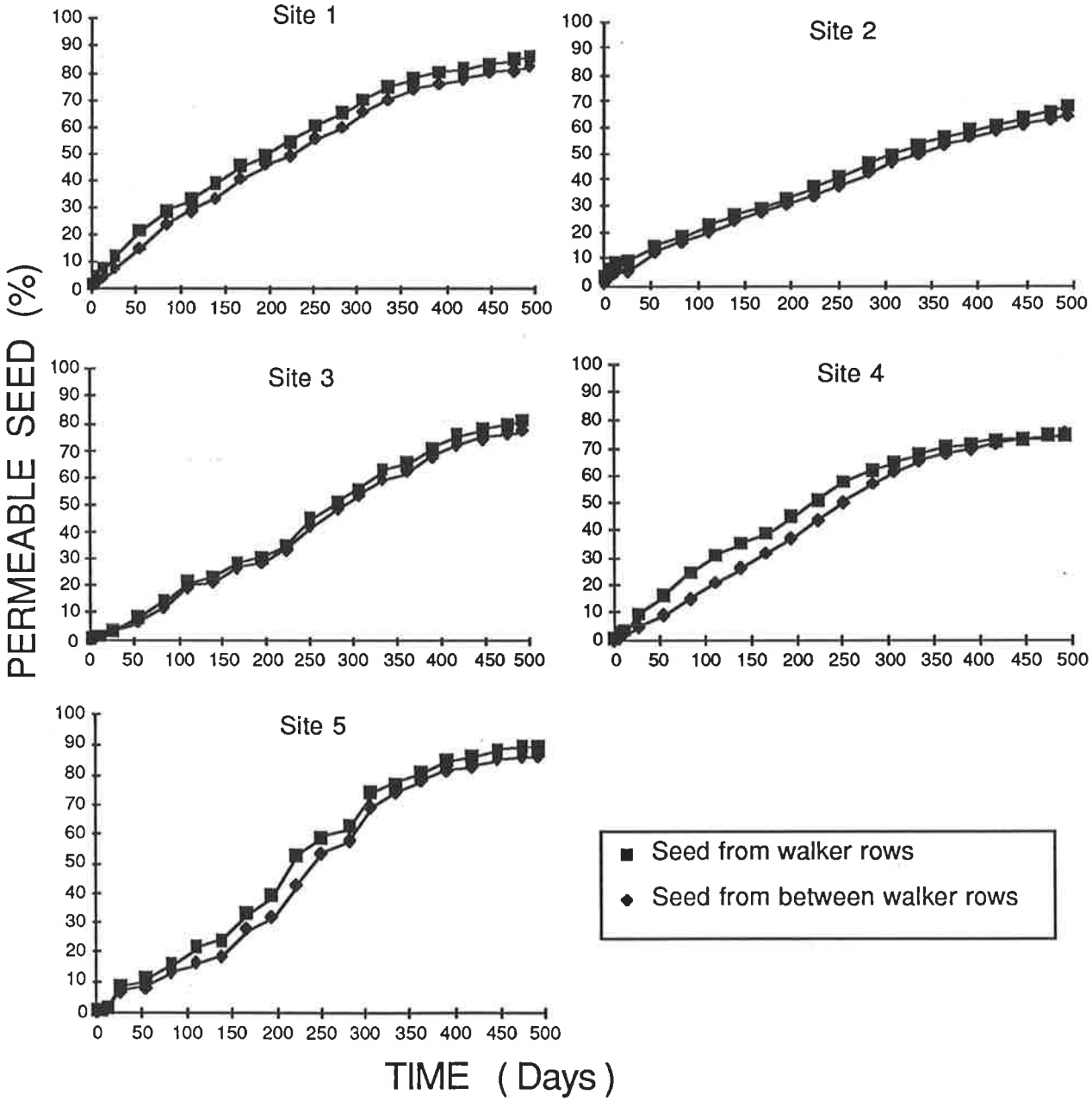


FIGURE 3.9: Field Survey 3 - cumulative germination of medic seed in the laboratory over 500 days. Seed was exposed to diurnally-fluctuating temperatures of 50/15°C except for 48-hour periods when germination percentages were assessed at 20°C.

PLATE 3.6: Field Survey 3 - Site 1 at Mallala in September 1985, showing differences in medic densities.

TOP - Walker row

BOTTOM - Between walker rows

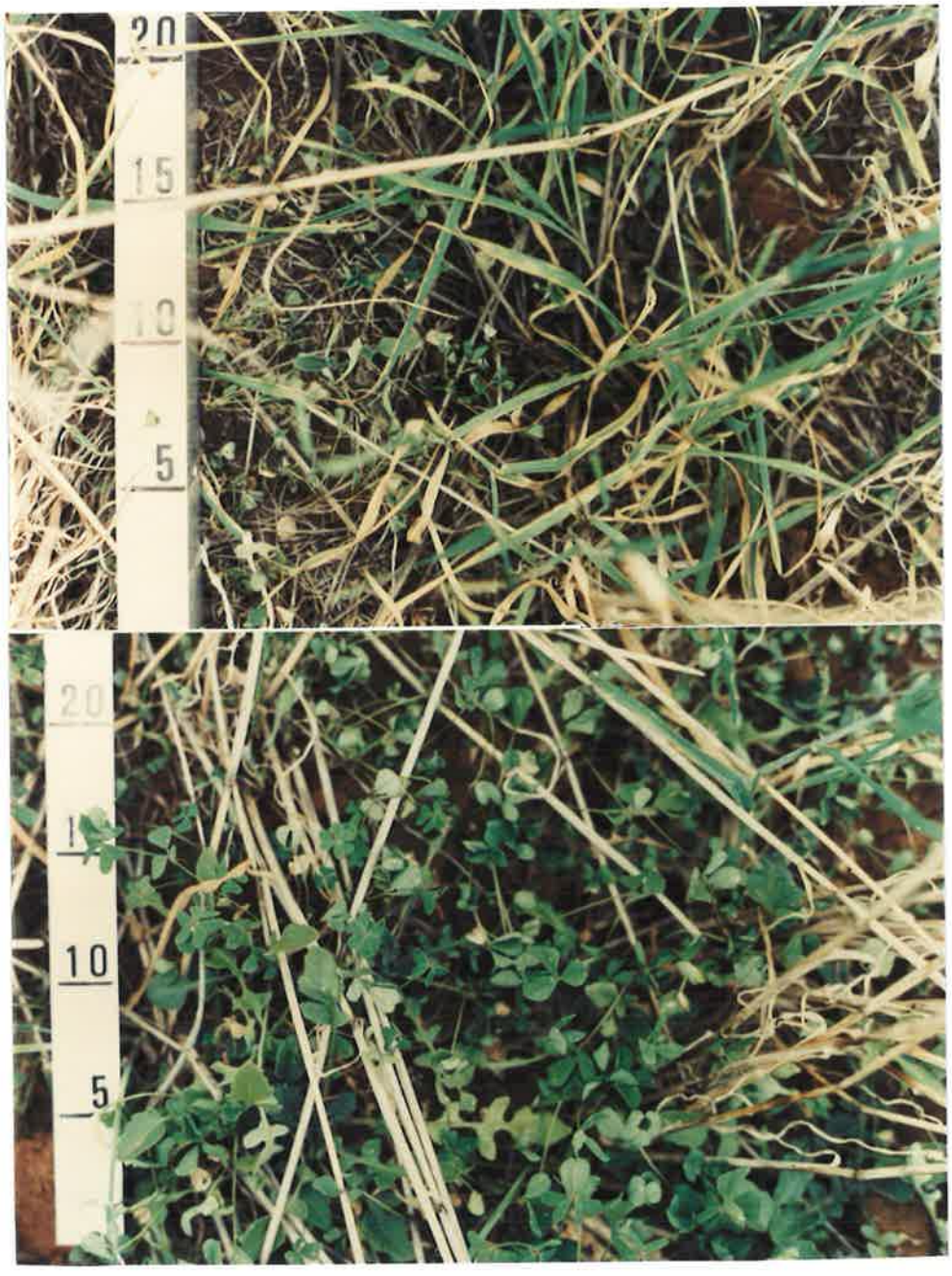

20
15
10
5
20
1
10
5

PLATE 3.7: Field Survey 3 - Sites 3 and 4 at Mallala in September 1985, showing differences in medic densities.

TOP - Site 3

LEFT - Walker row RIGHT - Between walker rows

BOTTOM - Site 4

LEFT - Walker row RIGHT - Between walker rows


20
15
10
5
20
15
10
5
20
15
20
15
10
5

3.5 Field Experiment 1: Effects of straw concentration on natural regeneration and productivity of an annual medic pasture at TwoWells

This field experiment was undertaken to more-closely examine the effects of cereal residues on the regeneration of medic pastures but at regular and more frequent intervals throughout the growing season than the observations made on the farm surveys. With this increased intensity of sample collection it was expected that changes in seed reserves and plant emergence and growth could be described in more detail. Imposition of a range of straw concentrations, as treatments, ensured collection of data on responses by medics to the full range of straw densities likely to be encountered in field situations.

The site chosen for the experiment was located at Two Wells, approximately 10 km south of Mallala and the climatic and edaphic factors were essentially the same as for the 1984 and 1985 survey areas.

3.5.1 Materials and Methods

A field experiment involving a range of imposed straw concentrations was commenced at Two Wells on the Adelaide Plains. Prior to the physical rearrangement of straw during the period February 3 to 6, 1984, the entire experimental area was covered with approximately 3900 kg/ha of Schooner barley straw which had been ungrazed. Seven straw densities approximately equivalent to 0, 500, 1000, 2000, 4000, 8000, 16000 kg/ha were imposed on plots 2 x 10 m by raking and removing or adding weighed quantities of the Schooner straw. Where it was necessary to remove large quantities of straw, the stubble was cut at a height of about 50 mm with a reciprocating mower. All plots were rolled using a rubber-tyred roller which effectively flattened the straw. The site was not grazed during the course of the experiment and the layout consisted of five blocks in a randomized block design. Photographs which show some of the techniques used to obtain these treatments are shown in Plate 3.8.

Sequential samplings at 28 day intervals commenced on February 14, 1984 and were repeated ten times. Straw dry matter concentration, medic plant density, medic dry matter production, and cereal (self sown) herbage dry matter production were measured.

Plate 3.8: Field Experiment 1 - operations during preparation of plot treatments at Two Wells.

TOP - Where it was necessary to remove straw it was cut with the reciprocating mower seen in the background. The quantities of straw either removed or added to plots were weighed in the field.

BOTTOM - All plots were rolled with a rubber -tyred roller to ensure that straw was flattened.

The northern half of each plot was divided into ten 1 x 1 m sections and one section was randomly allocated to each harvest time. A herbage and straw sample was collected from a 40 x 40 cm quadrat located within a section. The same was done on the southern half of the plot and data from both samples pooled, thus improving precision.

Two soil cores, to 5 cm depth, were collected with a Coile sampler at each sampling occassion from each harvested quadrat area, to determine medic seed reserves and to estimate the proportion of permeable seed. These cores were kept intact and each one placed in a plastic tub. Seven days after collection, the cores were watered to field capacity and maintained at 19°C for 14 days during which emerged seedlings were counted to give an estimate of permeable seed. This procedure minimized insect attack, ensured soil moisture was ideal and temperatures were optimum for medic germination. Two of the four soil samples from each plot were randomly selected and stored for eight weeks at a diurnally-fluctuating temperature between 19°C and 55°C to enable further breakdown of hard-seededness. The remaining soil samples were stored at 19°C for the 8 week period to act as a control. The germination procedure was then repeated, after which the remaining seed in all cores was extracted by dry sieving then counted.

On 19 May and 29 July 1984, soil temperatures were measured on two of the five replicates using two sensors (National Semiconductor, type LM 335) per plot, one buried immediately below the soil surface and the other 2.5 cm deep. Readings were made at hourly intervals from 0600 hours to 1800 hours each day.

Monthly rainfall records from Two Wells Post Office, 1 km from the experimental site, are shown in Appendix 1.

3.5.2 Results

Emergence of medic seedlings from soil cores

As only low numbers of seedlings emerged from soil cores taken from each straw concentration treatment during the period of the first five harvests the emergence data for this period have been pooled to enable more cogent comparisons of straw effects. At subsequent harvests there was emergence of seedlings in the field so these laboratory germination data were not pooled with earlier data. Emergence of seedlings from soil cores

moistened shortly after they were collected from the field ranged between 1.6 and 0.5 per cent of the viable seed in the cores (Figure 3.10) with the lowest emergence from soil cores previously covered by 8000 kg/ha of straw. This range is equivalent to medic densities of 32 to 10 plants/m^2 based on the mean seed reserve of 2300 seeds/m^2 at the site. These densities are similar to the actual medic densities later measured in the field i.e. 23 to 5 plants/m^2 (Figure 3.12). The emergence of seedlings from cores collected from the highest straw concentration treatment was higher than expected, otherwise there was a trend towards fewer seedlings emerging as straw concentration increased.

The densities of seedlings emerging from soil cores for each harvest occasion are shown in Figure 3.11. In February, 15 plants/m^2 emerged but temperatures in the field over the next month, promoted breakdown of hard-seededness so that 35 plants/m^2 were then found in the field. The changes over the following two harvests were not significantly different to results from Harvest 2 but there were fewer seedlings from cores collected at Harvest 5 and this situation continued over winter with negligible change in hard seed levels until Harvest 9 in spring when higher temperatures may have caused some additional breakdown of hard-seededness.

The general pattern of seedling emergence from soil cores during the second germination (Figure 3.10) was similar to the first germination, except the emergence from the 16000 kg/ha straw treatment was closer to the expected value, based on the trend of the other high straw treatments. The magnitude of values obtained in the second germination were about five times higher than values in the first germination showing that the artificial temperature environment (19-55°C) was more effective than the field environment in inducing increased seed permeability. After moistening the soil cores which were held at 19°C for 8 weeks, there was negligible emergence of seedlings which confirms the importance of thermal stimulus to breakdown of hard-seededness. These data are not presented here.

Analysis of variance indicated highly significant differences between straw concentration treatments in both the first and second germinations ($P<0.01$).

Plant density in the field

Medic plant density was not measured in the field prior to Harvest 6 (July 3) as there was insufficient rainfall to cause a general germination of medic seed. By this time, some seedlings had emerged but overall emergence was higher at Harvest 7. Plant senescence prior to Harvest 10 resulted in a reduction in plant density. Mean plant density over Harvests 6 to 10 has been calculated and a regression analysis undertaken using an exponential decay model with the concentrations of straw at Harvest 10 as the independent variable. This showed a strong and highly significant correlation between plant density and straw concentration in the field (Figure 3.12). The equation is as follows:

$$Y = 21.35e^{-0.00049X} \quad 100r^2 = 95.6 \text{ ***}$$

where Y is mean medic plant density (#/m^2) over the period between Harvests 6 and 10 and X is straw concentration (kg DM/ha) at Harvest 10.

Dry matter yield of medic

The dry matter yield of medic was measured at Harvests 7, 8, 9 and 10 but the plants were stunted in growth throughout the experiment, moreover there was a high degree of variability in the data collected at each harvest. The cause of the stunting was not determined but competition from self-sown barley, evenly distributed over the site, may have contributed to the poor growth of medic. In order to reduce variability, the mean dry matter production from Harvests 7 and 8 have been pooled for each treatment and the means were also obtained for Harvests 9 and 10. In Figure 3.13 the mean values have been plotted against the straw concentration at Harvest 10. The following equations were obtained by linear regression:

$$Y=0.569e^{-0.000553X} \quad r^2=0.83 \text{ **}$$

where Y is mean dry matter yield (gDM/m^2) of medic from Harvests 7 and 8 and X is straw concentration (kgDM/ha) at Harvest 10.

and $Y=3.121e^{-0.000387X}$ $r^2=0.85$ **

where Y is mean dry matter yield (gDM/m^2) of medic from Harvests 9 and 10 and X is straw concentration (kgDM/ha) at Harvest 10.

Seed reserves in the soil

There was little change in the seed reserves in the 0-5 cm of soil until Harvest 6 then there was a relatively sharp fall presumably due to germination, finally the density of seed increased at Harvest 10 due to production of new seed by the maturing medic plants (Figure 3.14). Straw had no effect on the seed reserves but there was a significant ($P<0.01$) difference in seed density depending on harvest time (LSD [P=0.05]= 360 seeds/m^2). Although the drop in seed numbers between Harvest 5 and 8 corresponds with the appearance of seedlings the magnitude of the respective changes were very different, so that some 450 seeds/m^2 were lost. Pathogen attack or desiccation prior to emergence, and seed at excessive depths to allow emergence are possible causes for this loss but no direct evidence was collected. The mean seed number in soil cores was close to 20/core, equivalent to 2300 seeds/m^2, but the number of seedlings established represented less than one percent of the seed present.

Breakdown of straw in the field

The values of straw concentration measured in the field at each harvest are shown in Figure 3.15. Photographs of typical straw densities at the commencement of the experiment on February 6, 1984, are shown in Plates 3.9. and 3.10. There was considerable effort required to remove as much barley residue as possible from the nil-straw plots but it was impracticable to remove all traces of the previous barley crop. Furthermore the wind moved some of the residues back onto the Nil treatments, thus the concentration of straw measured on these plots was approximately 500 kg/ha shortly after the experiment began.

The largest absolute changes in straw concentration occurred where initially the straw concentrations were highest. The percentage decrease in straw concentration was unaffected by the initial straw concentration and the mean percentage change was 59.3 per cent between Harvest 1 and Harvest 10.

Soil temperatures

The soil temperatures graphed in Figure 3.16 and Figure 3.17 for May 19 and Figure 3.18 and Figure 3.19 for July 29 show that the diurnal temperature fluctuations were reduced as straw concentration increased. At the soil surface, the highest straw concentration reduced maximum soil temperature by approximately 2°C and increased the soil minimum temperature by the same amount, relative to the lowest straw concentration. Maximum and minimum temperatures at 2.5 cm depth were only approximately 1°C less than at the surface. The temperature fluctuation (difference between daily max. and min.), for each straw concentration treatment, have been plotted in Figure 3.20 and indicate curvilinear trends.

3.5.3 Discussion

In farm surveys it was often difficult to precisely determine the effects of straw at low concentrations owing to high levels of variability in pasture density or productivity, probably caused by interactions with factors including broadleaf weeds and grazing by stock or insects. In this experiment there were few broadleaf weeds present and the area was not grazed, therefore the data represents the effects of straw concentrations more exactly. The relationships between medic plant density or medic dry matter yield and straw concentration (shown in Figures 3.12 and 3.13) indicate that the most rapid changes in medic occurred at low straw concentrations. These trends closely agree with the results found in the three previous paddock surveys.

The trends of seedling emergence from soil cores shortly after collection from the field show that the amount of permeable seed was a major influence on seedling density in the field. At the highest straw concentration (16000 kg/ha), however, the quantity of mulch above the soil may have physically impeded emergence of seedlings through the straw; seedling density was lowest in this treatment although seedling emergence in soil cores indicated the presence of a relatively high density of permeable seed.

The high density of seedlings which emerged from the highest straw treatment during the first germination was very unexpected but this did not persist in the second germination (Figure 3.10). Some other factor/s beside temperature may have been responsible for the

initial high level of permeable seed, possibly a chemical effect or action of soil microorganisms. During the breakdown of straw in the highest straw concentration there was considerable decay of residue at the soil-straw interface and the straw was approximately 10 cm thick which maintained moist conditions below it. Anaerobic fermentation could have proceeded under these conditions with liberation of short chain fatty acids as breakdown products. If such acids came in contact with seeds of annual medic there is a chance that the seed coat could have been rendered permeable by chemical action. The emergence of seedlings in the second germination followed the pattern set by the first germination, with the exception of that at the highest straw concentration treatment. The hard seed remaining after the first germination may have remained preconditioned by temperature regimes experienced in the field which would explain why the patterns in the two germinations were so similar. The different seed behaviour in the highest straw treatment, when both germinations are compared, suggests that the stimulus for the initial germination was transitory. This observation supports the theory of chemical breakdown of hard-seededness in this case.

The influence of temperature on plant development of subterranean clover and annual medic have been studied extensively (Morley 1958; Kleinig 1965; McWilliam *et al.* 1970; Cocks 1973; Greenwood *et al.* 1976; Adem 1977; Silsbury *et al.* 1984). Silsbury *et al.* (1984), working with subterranean clover cv. Mt Barker, found that temperature had relatively small effect on nitrogen fixation measured by acetylene reduction, with nitrogenase activity highest at 10°C. Similarly the growth rates of plants were not very sensitive over the temperature range of 15-25°C which supported earlier conclusions of Fukai and Silsbury (1976). The temperature differences caused by the range of straw concentrations over autumn and winter in the present studies were only small and are unlikely to be responsible for much variation in pasture growth rate. Soil temperatures were not monitored in the field during the germination phase of seedling development. The mulch treatments would have had an effect on soil temperatures at this time and these temperatures could have had modest effects on seed germination and establishment.

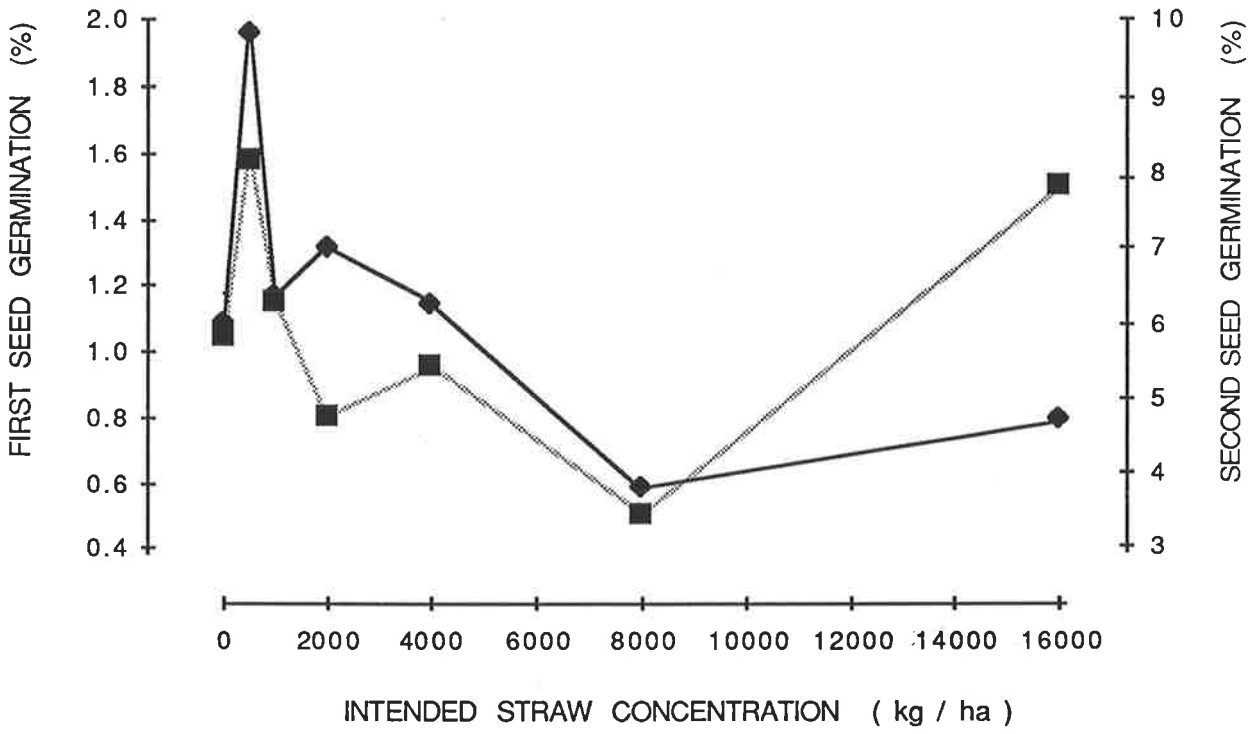


FIGURE 3.10: Seed germination percentages in soil cores taken from beneath a range of straw concentration treatments. The first germination was from soil cores directly from the field and the second germination was after storage of soil cores at 55/19°C (day/night) for eight weeks. Values are the means of the first five harvests.

LSD (P=0.05) first germination = 0.7 per cent

LSD (P=0.05) second germination = 3.2 per cent

First germination

Second germination

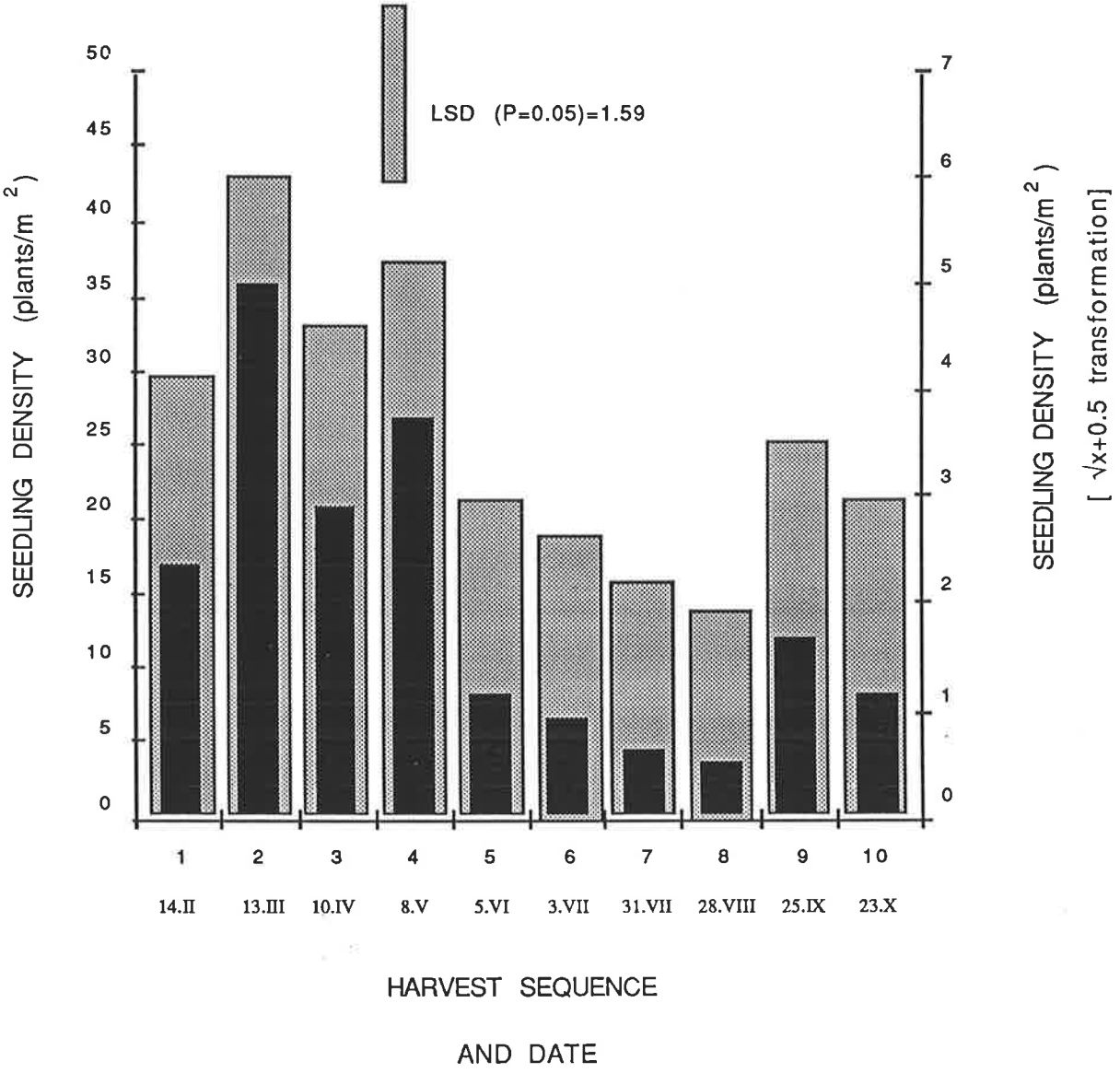


FIGURE 3.11: Seedling densities in soil cores after the first laboratory germination, averaged over all straw treatments for Harvests 1 to 10.

Transformed data

Mean after analysis, retransformed

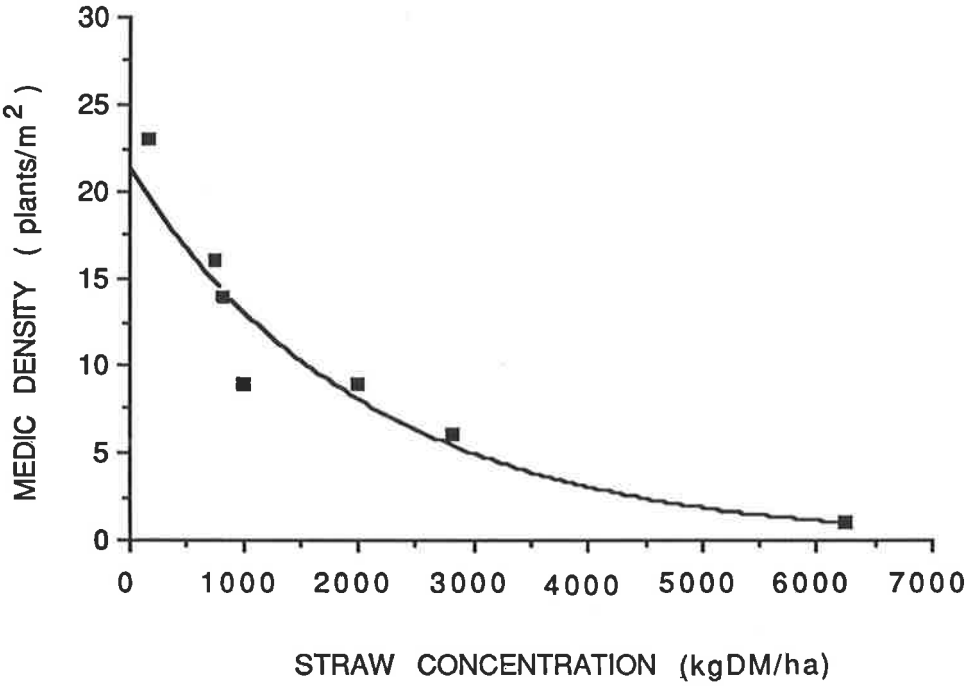


FIGURE 3.12: Medic plant densities in the field (mean of Harvests 6 to 10) at each straw concentration (measured value) at Harvest 10 and the plot of the equation $Y=21.35e^{-0.00049X}$ fitted to the data.

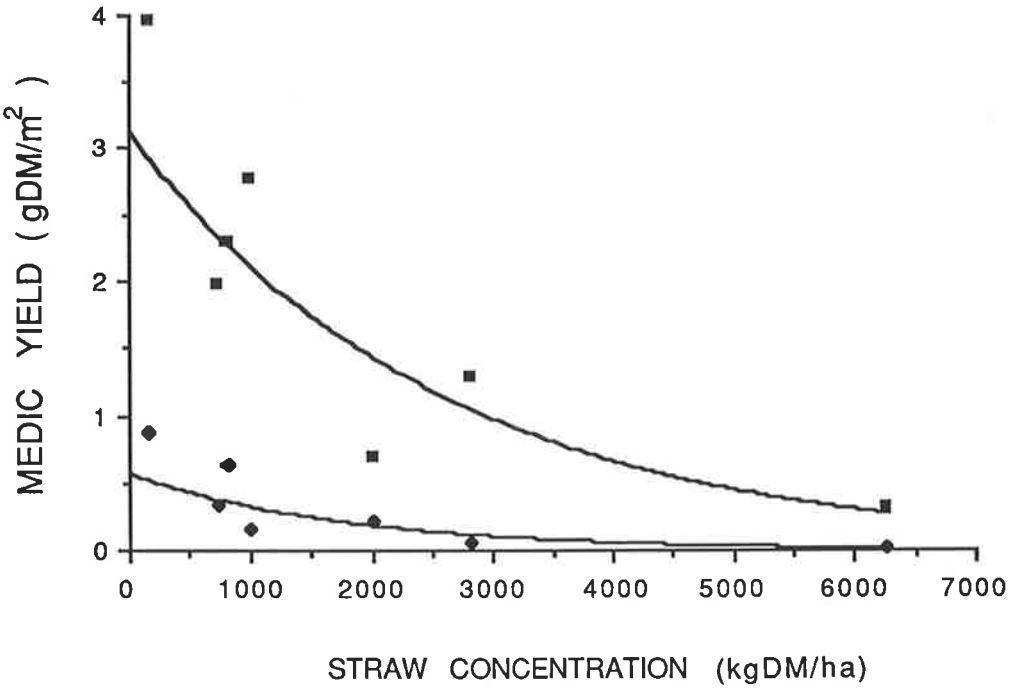


FIGURE 3.13: Medic dry matter yield plotted against straw concentration at Harvest 10.

- Mean of medic yields at Harvests 7 and 8 (July 31 and August 28)
- Mean of medic yields at Harvests 9 and 10 (September 25 and October 23)

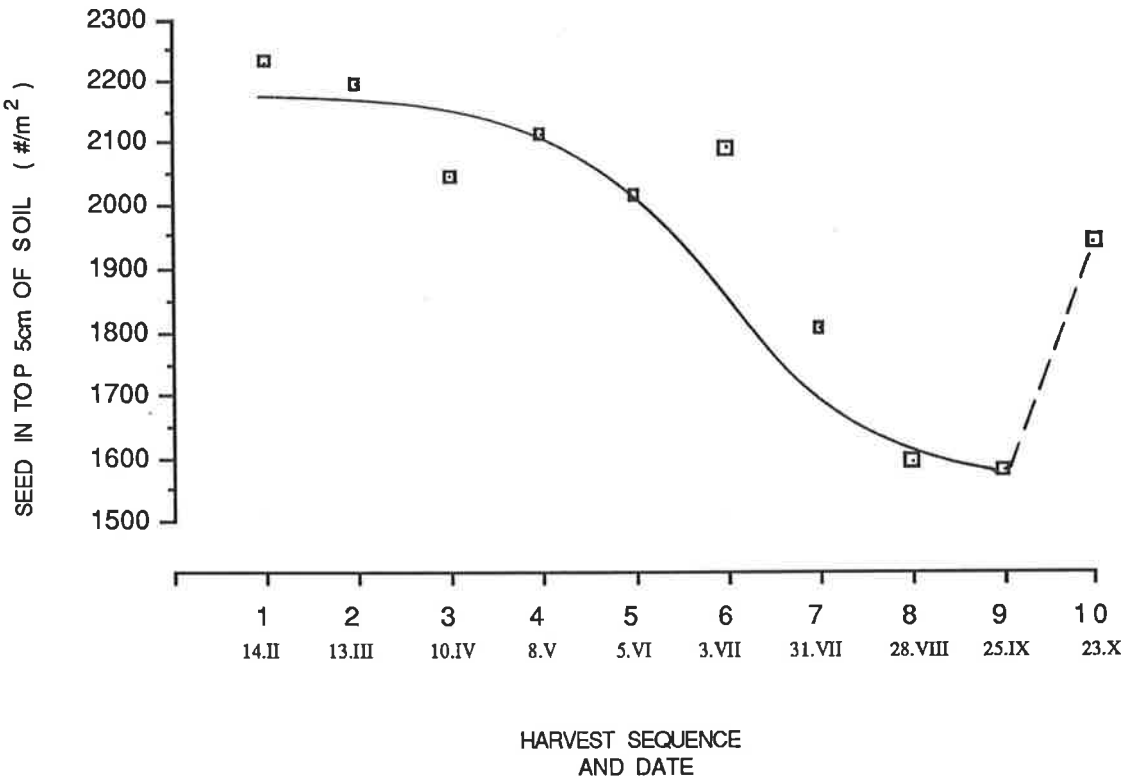


FIGURE 3.14: Medic seed reserves in the top 5 cm of soil on various occasions throughout the growing season. Line of best fit drawn by eye. Reduction between Harvest 5 and Harvest 8 corresponds with seed germination over autumn and winter. The increase at Harvest 10 was due to new seed production in spring.

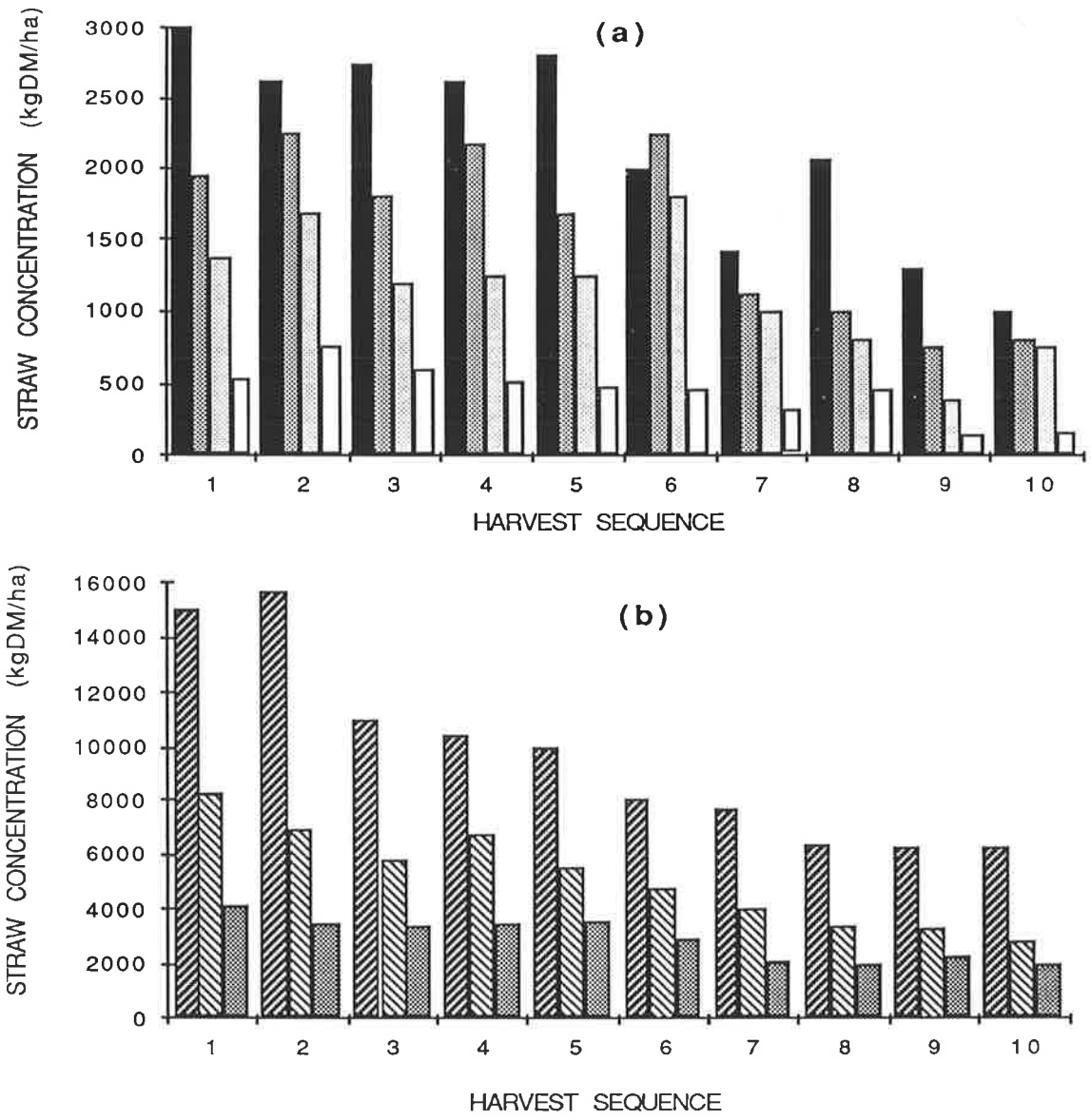
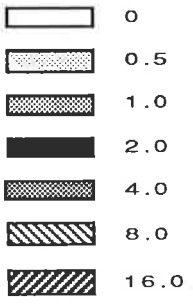


FIGURE 3.15: Straw concentrations measured in the field during the experiment showing the marked decline with time.

(a) Straw concentration treatments- nil, 0.5, 1.0, 2.0 t/ha

(b) Straw concentration treatments- 4.0, 8.0, 16.0 t/ha

Legend Straw treatments (t/ha)



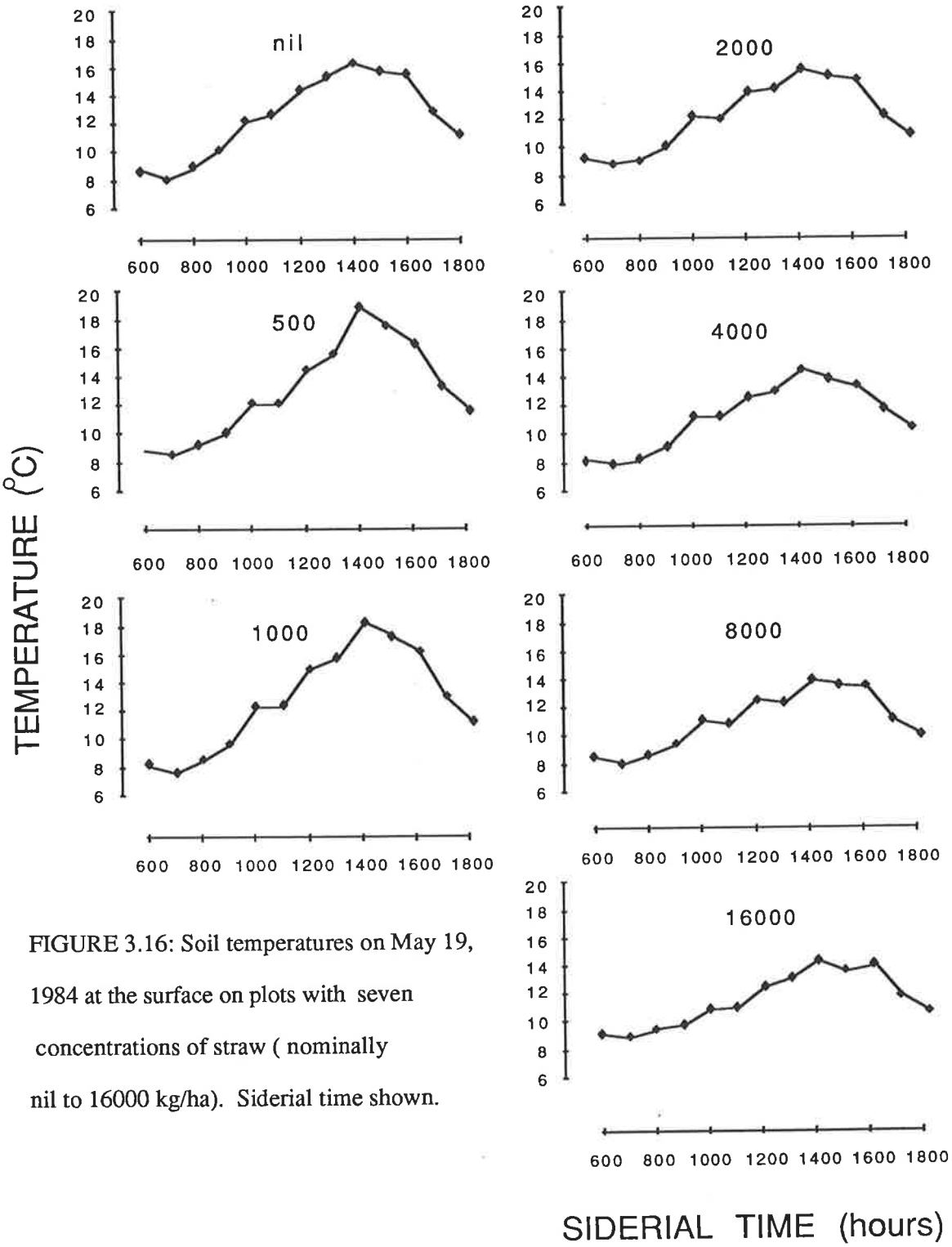


FIGURE 3.16: Soil temperatures on May 19, 1984 at the surface on plots with seven concentrations of straw (nominally nil to 16000 kg/ha). Siderial time shown.

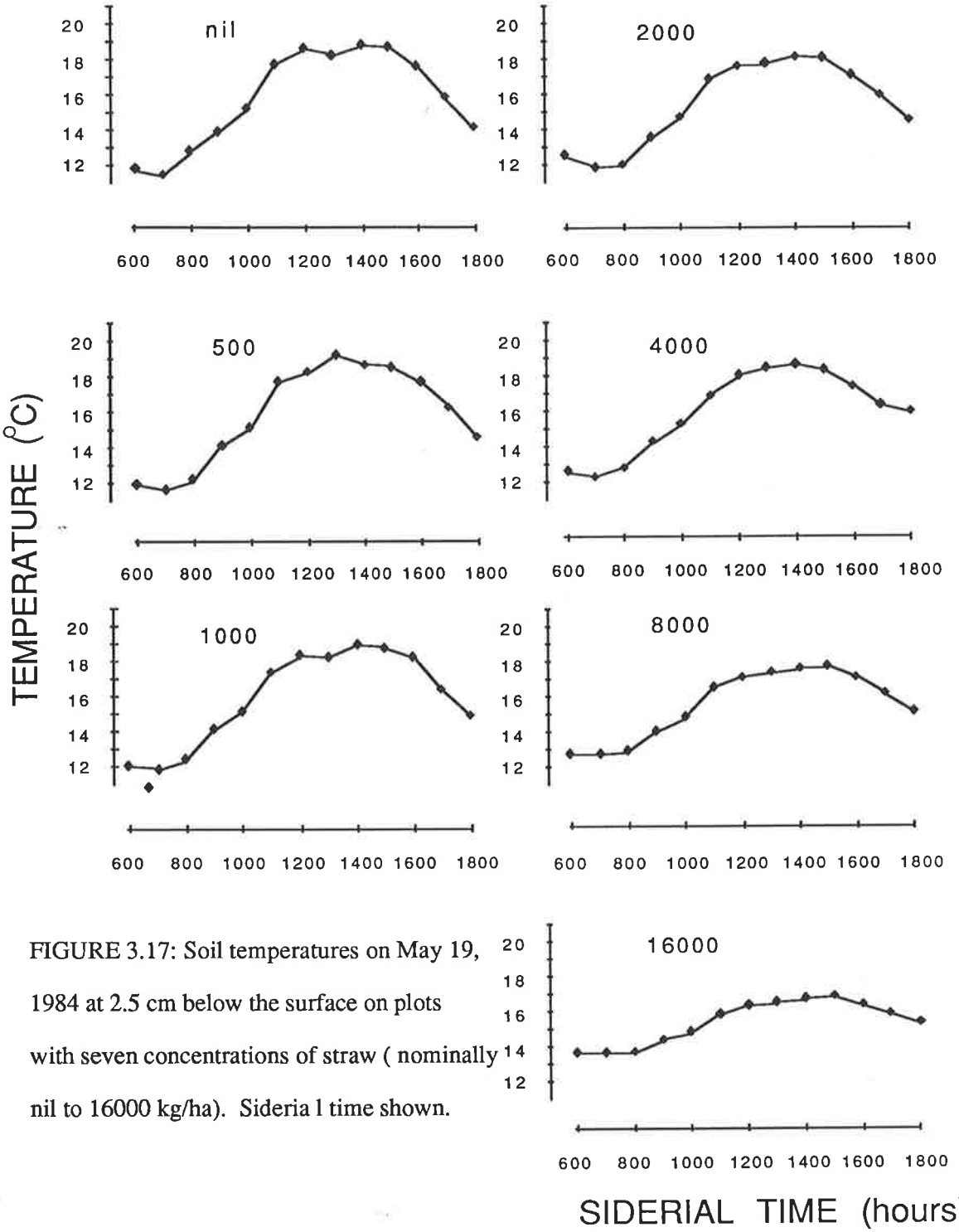


FIGURE 3.17: Soil temperatures on May 19, 1984 at 2.5 cm below the surface on plots with seven concentrations of straw (nominally nil to 16000 kg/ha). Sideria l time shown.

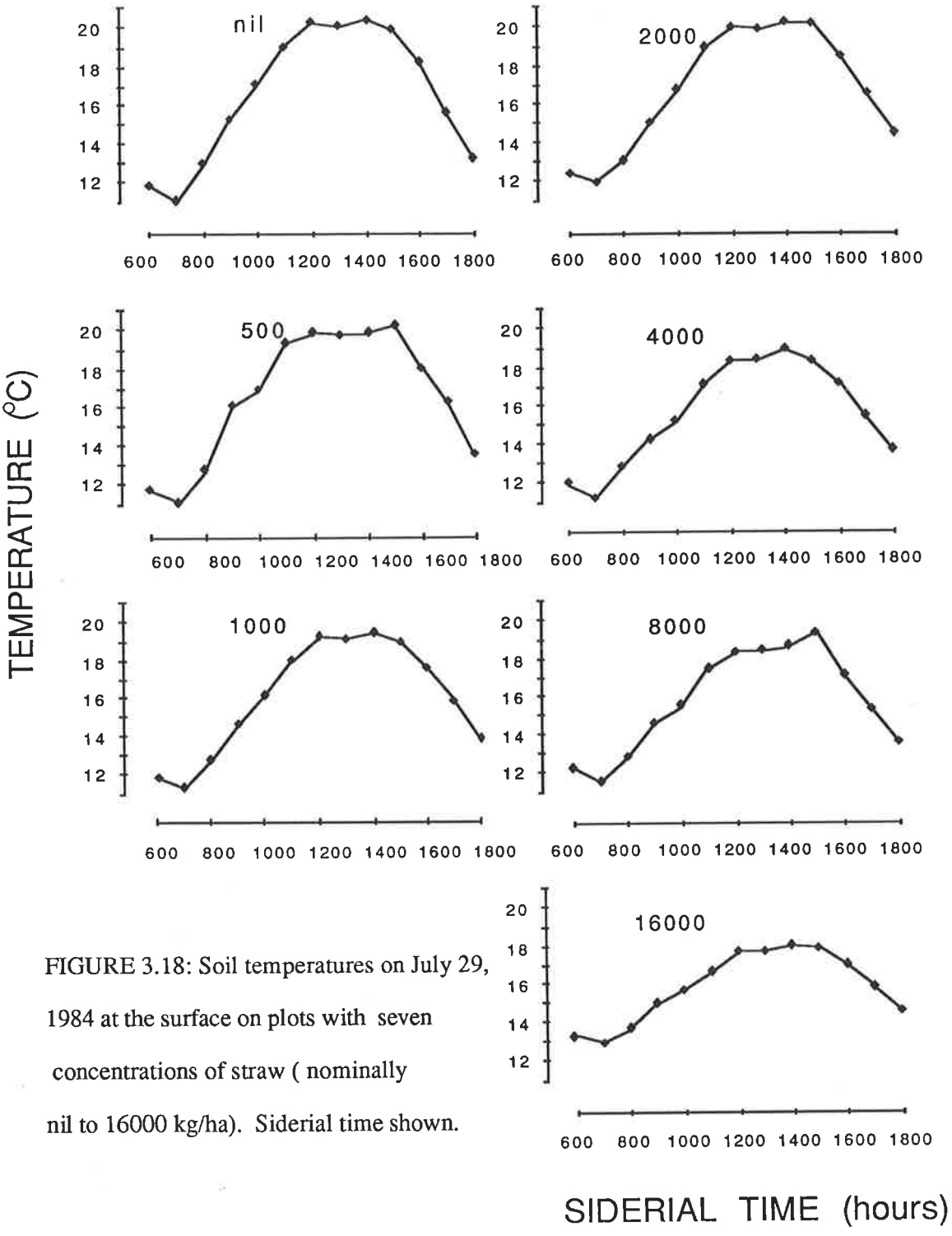


FIGURE 3.18: Soil temperatures on July 29, 1984 at the surface on plots with seven concentrations of straw (nominally nil to 16000 kg/ha). Siderial time shown.

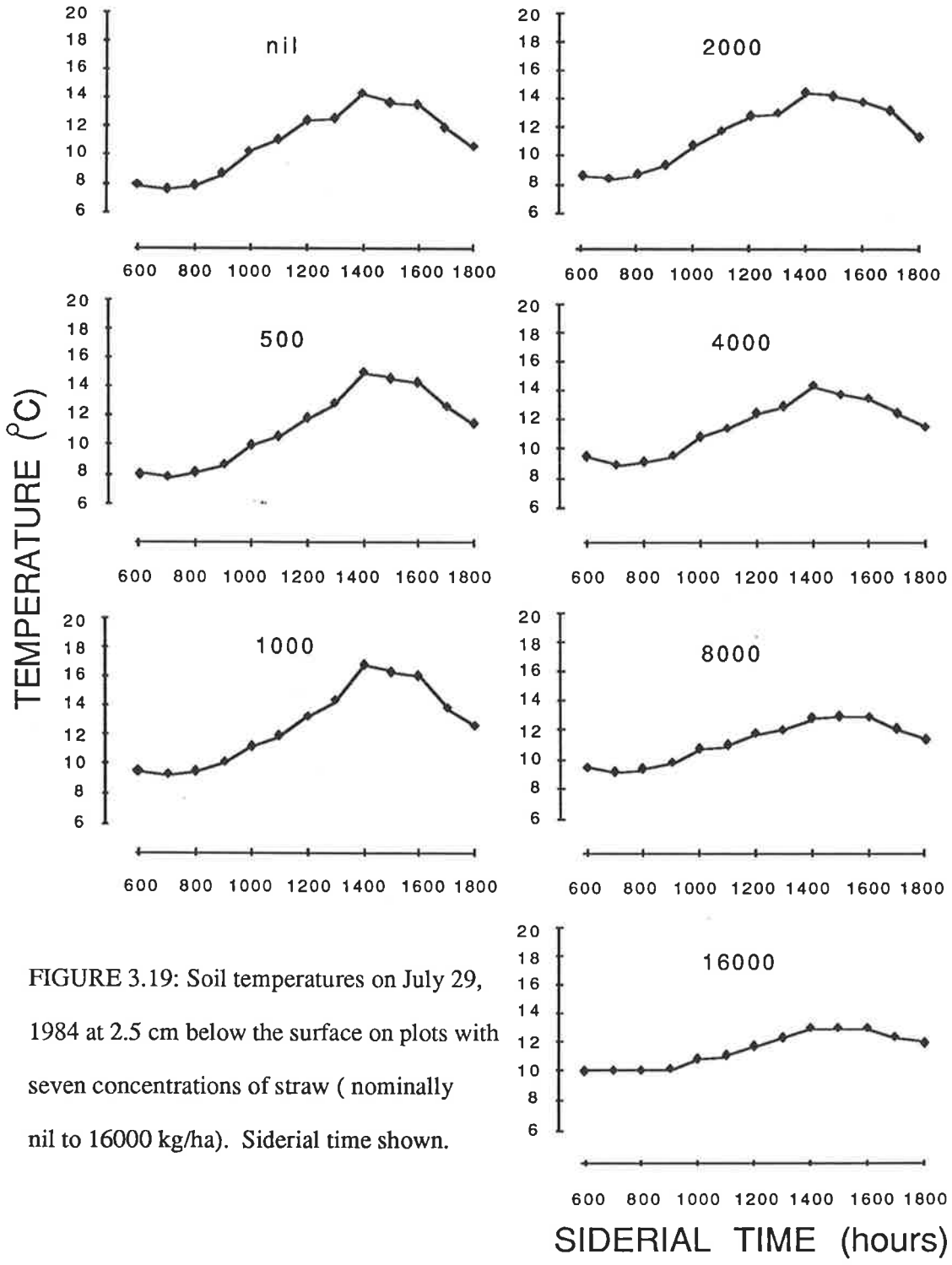


FIGURE 3.19: Soil temperatures on July 29, 1984 at 2.5 cm below the surface on plots with seven concentrations of straw (nominally nil to 16000 kg/ha). Siderial time shown.

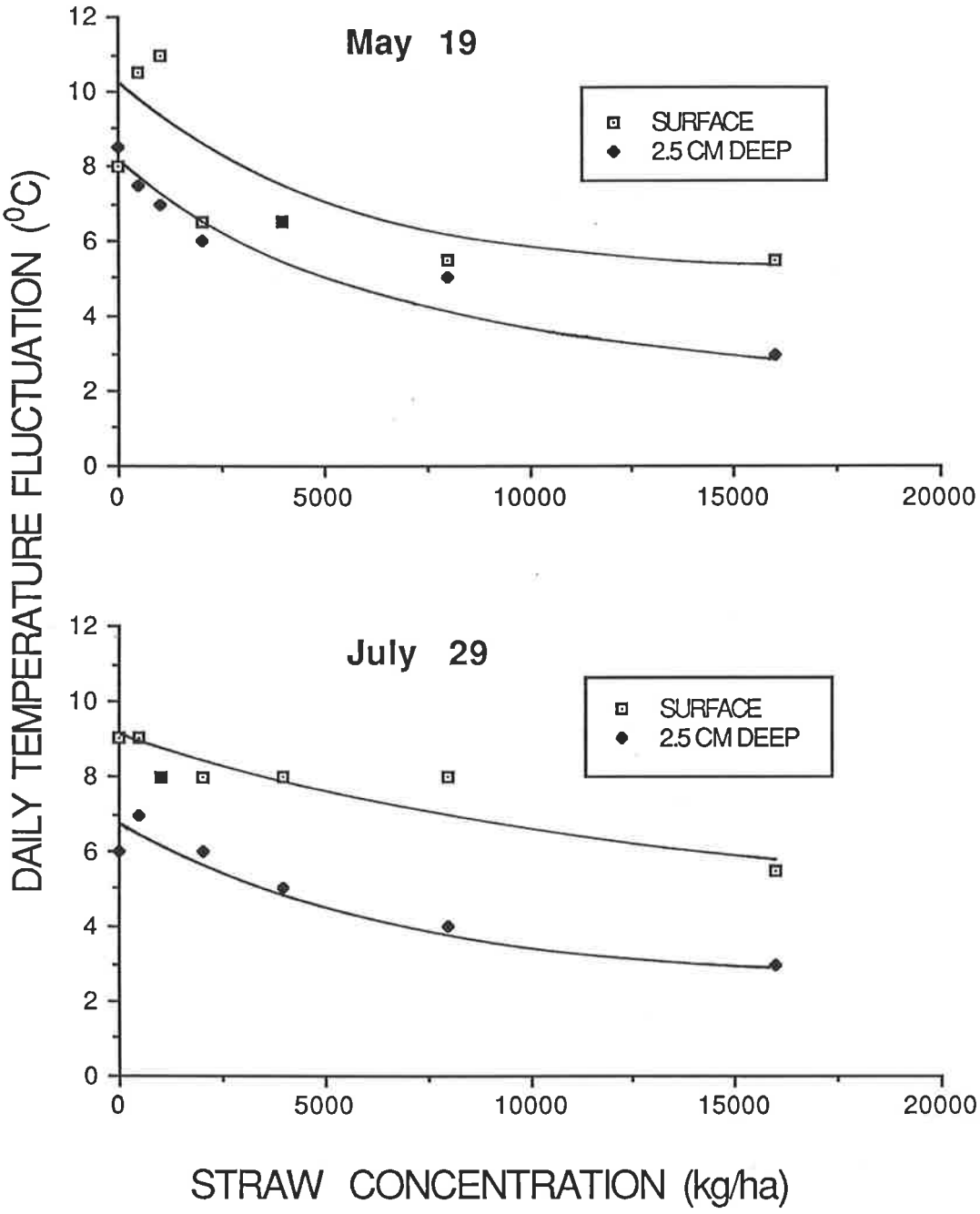


FIGURE 3.20: Daily temperature fluctuations in the soil beneath a range of concentrations of cereal straw residue.

PLATE 3.9: Field Experiment 1 - typical straw concentrations at the beginning of the Two Wells experiment, February 6, 1984. Plots lettered in order of treatments in the first block. Actual values of straw concentrations (as measured) were slightly different to the intended treatment concentrations.

Plot	Treatment
D	Nil
G	1000 kg/ha straw
C	2000 kg/ha straw
F	4000 kg/ha straw

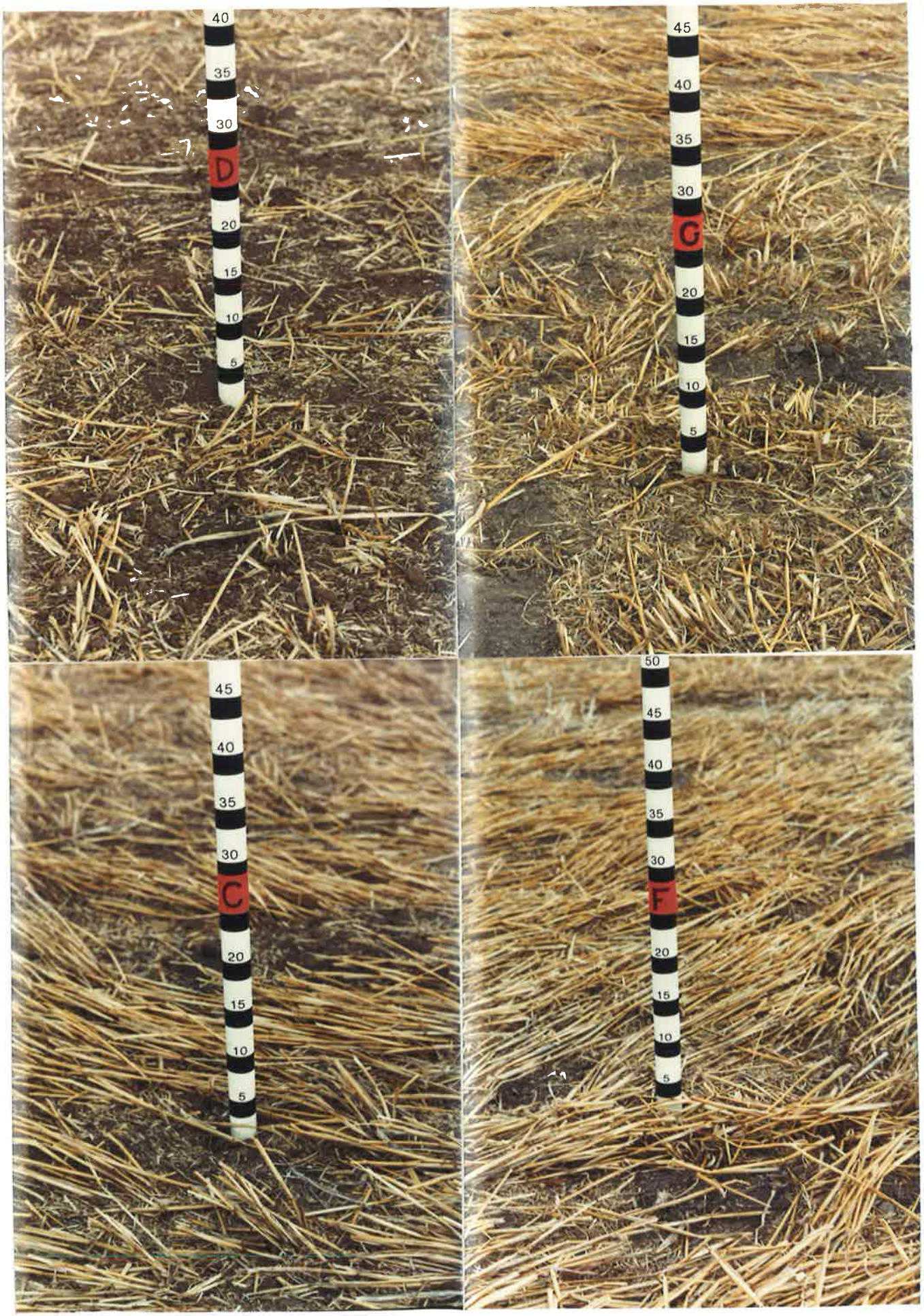

40
35
30
D
20
15
10
5
45
40
35
30
G
20
15
10
5
45
40
35
30
C
20
15
10
5
50
45
40
35
30
F
20
15
10
5

PLATE 3.10: Field Experiment 1 - typical straw concentrations at the beginning of the Two Wells experiment, February 6, 1984. Plots lettered in order of treatments in the first block. Actual values of straw concentrations (as measured) were slightly different to the intended treatment concentrations.

Plot	Treatment
B	8000 kg/ha straw
E	16000 kg/ha straw


55
50
45
40
35
30
B
20
15
10
5
55
50
45
40
35
30
E
20
15
10

3.6 Field Experiment 2: Effects of straw concentration on emergence of annual medic seedlings in the field at Reeves Plains

A field experiment was commenced during early winter in order to examine the effects of different concentrations of barley straw (chaffed) on emergence and early seedling development of barrel medic (*Medicago truncatula*) cv. Paraggio. Commercial scarified seed, which had a low percentage of hard seeds (2.4%), was sown to avoid possible confounding effects of straw cover on breakdown of hard seed and also it ensured a consistent density of permeable seed in all treatments which was especially necessary because of the small size of treatment areas. This experiment also provided an opportunity to assess the importance of physical impedance by a packed mulch of cereal chaff to seedling emergence. Any phytotoxicity exhibited by the fresh cereal straw mulch could be observed more closely in this experiment than in either Field Experiment 1 or the field surveys because of the increased frequency of observation during this experiment. Additionally, the experiment enabled a close inspection of developing seedlings for evidence of pathogen attack, though it was recognized that a single experimental site would not give an adequate indication of the general importance of disease as a factor in seedling establishment.

Poor re-establishment of legume pastures has frequently been attributed to pathogen attack (McKee and Kellock 1974; Stovold 1974; Barbetti and MacNish 1983; Bretag 1985) and root rots of pasture legumes, especially of subterranean clover, are widespread in southern Australia (Barbetti *et al.* 1986). Bretag (1985) surveyed the incidence of pathogenic fungi over large tracts of the cereal-sheep zone of Victoria where he found the most important pathogens on roots of medic to be *Pythium irregulare*, *Rhizoctonia solani* and *Fusarium acuminatum* because they were common and strongly pathogenic. Barbetti *et al.* (1986) also included *Phytophthora clandestina* as an important pathogen of root rot of pasture legumes. It should be noted that many *Pythium* species have a large capacity to degrade cellulose and pectin (Hendrix 1974), they are therefore able to colonize and rapidly multiply on cereal residues. Incorporation of residues of mature cereal crops into soil has been shown to increase the propagule density of *Pythium*

spp. (Singh and Pandy 1966), and the order of effectiveness was barley straw> oat straw> pea stover> wheat straw. The carbon to nitrogen ratio of the crop residue may affect the level of disease severity according to Okpala (1975) but Singh and Pandy (1966) found no such relationship in their studies.

3.6.1 Materials and Methods

The experiment was located in a field of barley stubble at Reeves Plains, 50 km north of Adelaide. Analysis of soil samples collected prior to commencement of the experiment indicated that few medic seeds were present, therefore medic plant density from these seeds was inconsequential. Daily rainfall recorded at Mallala Post Office, the closest observation point, is shown in Appendix 2.

On June 25, 1986, perspex rings 10 mm deep and 125 mm diameter were pressed into the soil surface which was damp at the time. The soil was excavated from within each ring and sieved through a 3.35 mm square-mesh sieve. The seed bed within the ring was lightly scarified with a knife and 40 seeds of barrel medic (*Medicago truncatula)* cv. Paraggio were sown in a random pattern in each excavation after which the sieved soil was returned and lightly compacted to leave the soil surface at its original level. This provided a seed density of 3250/m^2. A weighed quantity of Galleon barley straw (chaffed in a Wiley mill) was then placed on the soil surface through a 205 mm diameter, bottomless bucket and levelled leaving a cylindrical cover of straw above the seeds. After removing the bucket the microplots were irrigated with approximately 100 ml of rain water (equivalent to 3 mm of rainfall) by means of a watering can to compact and bond the straw and minimize movement of straw by wind. Rainfall shortly afterwards thoroughly consolidated the mulches so there was no observable loss of material during the experiment. Sheep were excluded from grazing the experimental area by covering the site with steel mesh exclosure cages. The experimental design consisted of nine straw treatments ranging in concentration viz. 0, 2000, 3000, 3500, 4000, 4500, 5000, 6000 and 8000 kg/ha of chaff, randomized in eight blocks. Straw treatments were in contiguous pairs in each block and data from each pair were pooled. Four blocks of the experiment are illustrated in Plate 3.11.

PLATE 3.11: Field Experiment 2 - microplots in the field at Reeves Plains, June 1986. Ends of the exclosure cages were closed later to prevent grazing by sheeep.

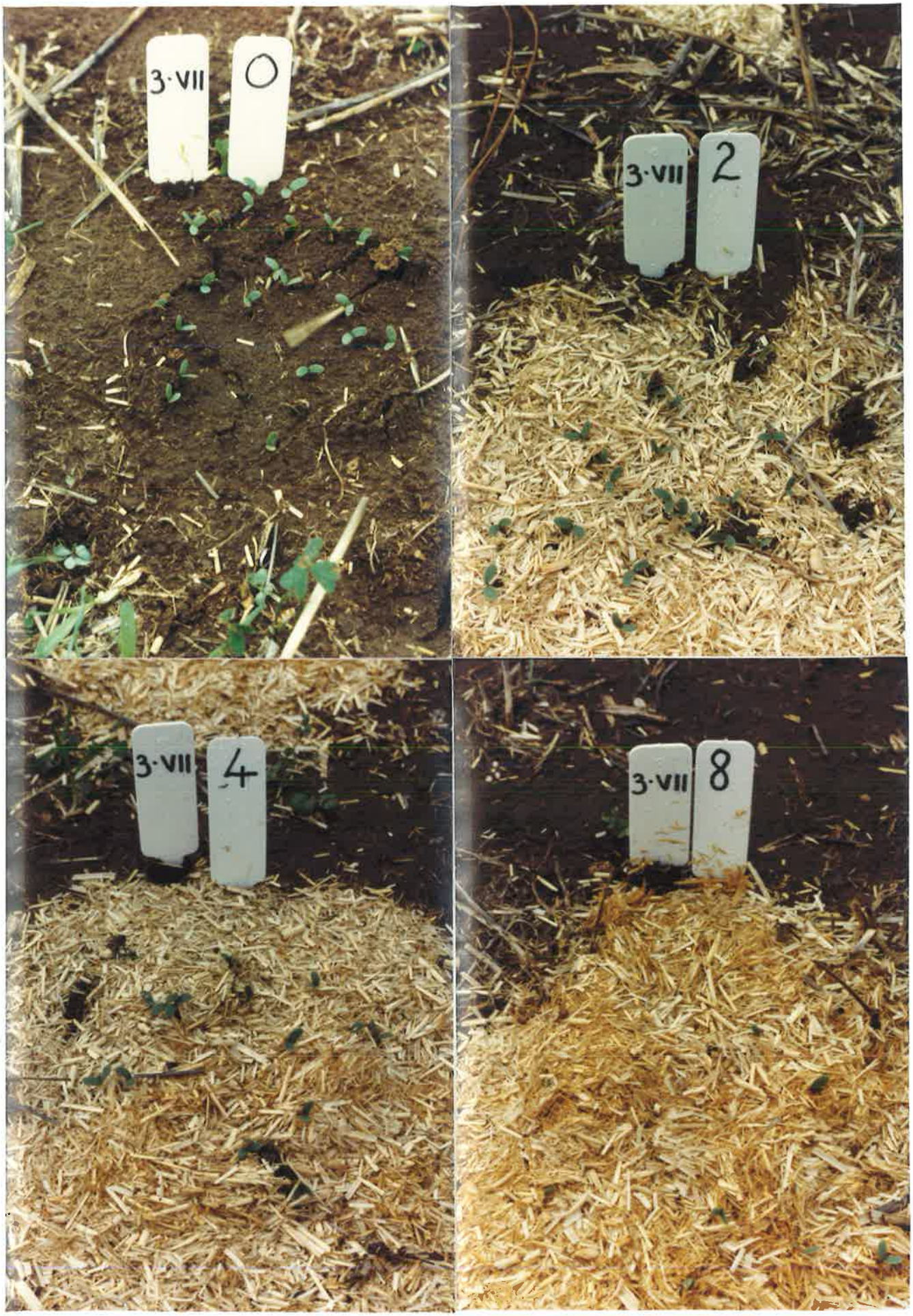
Seedling emergence counts were made once per week for the first three weeks after sowing and then at 48 days after sowing when medic seedlings were harvested by cutting at ground level with a scalpel. Harvested herbage was dried at 80°C for 24 hours then weighed. Disturbance of protective exclosure cages on four replicates and consequent grazing of microplots by sheep limited data collection to only four replicates 48 days after sowing.

3.6.2 Results

The concentration of chaff cover over the microplots had a considerable impact on medic seedling emergence, one week after sowing. Photographs of representative microplots, at this time, are shown in Plate 3.12. Regression analysis, using a third order polynomial, was used to determine the relationship between seedling density and chaff concentration. Graphical representation of the equation fitted to the data is shown in Figure 3.21 and 96.9 per cent of the variation in medic plant density was explained by changes in chaff concentration. This initial impedance to seedling emergence by the chaff (first seven days) was not maintained: seedling densities were all equal i.e. 31.6±0.9, 14 days after sowing. The final mean density of medic plants 48 days after sowing was 32.3±1.1 per microplot with no differences between treatments. Thus emergence averaged some 82 per cent of potential. The mean dry matter yield of medic taken from the microplots was 1.01±0.09 g and there were no significant differences between treatments.

Those seedlings not harvested for dry matter yields enabled investigation of the presence of pathogens on the young plants. There were necrotic lesions on the stems of seedlings sampled from the areas with the highest concentration of chaff but there were no lesions present on seedlings taken from chaff-free plots. A detailed quantification of the presence of lesions was not attempted. These lesions were examined by a plant pathologist (Dr A. Dube, South Australian Department of Agriculture) who made a positive identification of *Phoma* sp. as the cause of the necrosis.

PLATE 3.12: Field Experiment 2 - comparisons of medic seedling emergence in four of the treatments, seven days after seed was sown at Reeves Plains. Note the progressive reduction in medic seedling densities as straw-mulch concentration increased (0, 2, 4 and 8 t/ha shown).


3·VII 0
3·VII 2
3·VII 4
3·VII 8

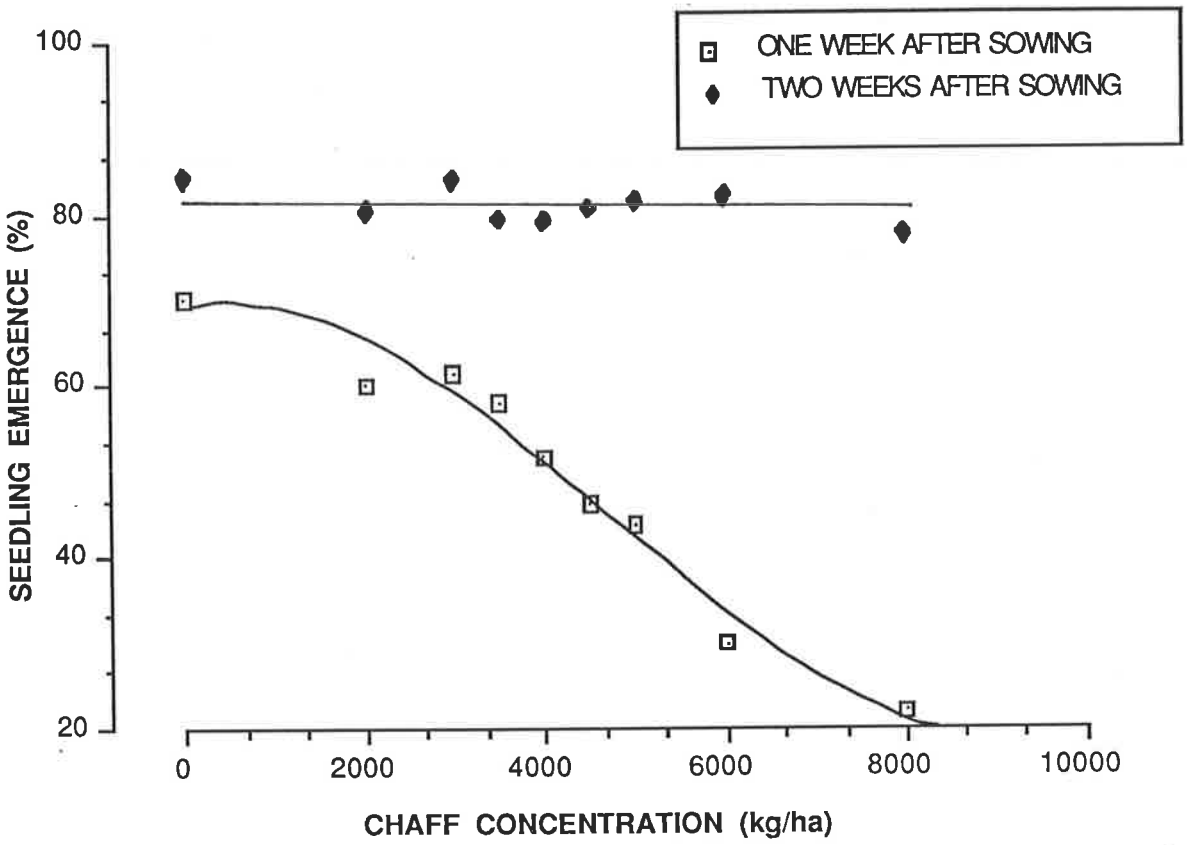


FIGURE 3.21: The effect of chaff concentration on medic seedling emergence in the field, one and two weeks after sowing. The regression equation fitted to the one week data was $Y=69.22+2.172X-2.316X^2+0.162X^3$ $R^2=0.969$ ***

3.6.3 Discussion

This experiment demonstrated that seedling establishment in the field may be unaffected by quantities of straw mulch which covered the soil. These results differed greatly from the variation found in previous investigations (Field Survey 1, 2,3 and Field Experiment 1), where effects of straw on hard-seededness were present. The higher densities of chaff physically retarded the emergence of seedlings through the chaff and the seedlings remaining below the chaff must have been under stress. However, this induced stress was insufficient to permanently inhibit development of seedlings as they eventually emerged through the straw and there were no lasting effects on dry weight yield of medic, measured seven weeks after sowing.

Presence of mulch did not affect the incidence of pre-emergence pathogen attack, possibly because pathogenic fungi were not active at this site during the course of the experiment. The propagule density, activity and pathogenicity of many microorganisms in the soil are very dependant on temperature, moisture, and other edaphic factors; especially for fungi such as *Pythium* spp. (Stanghellini 1974; Bretag 1985). Soil temperatures and soil water potentials during the experiment may not have been conducive to activity of root rot pathogens. Additionally, certain of these pathogens are extremely sensitive to competition from less pathogenic microorganisms: for example, *Pythium* is primarily an opportunistic pioneer colonizer of living, dying, or dead substrate and can be rapidly overtaken by secondary saprophytes. The presence of *Phoma* on the stems of seedlings was not a serious disease as plant development did not seem to be affected. Barbetti (1986) studied the colonization of subterranean clover by fungi associated with leaves and petiole and concluded that *Phoma medicaginis* was a pathogen of little consequence under field conditions although it was able to infect seedlings as early as one week after emergence. He noted that outbreaks of disease in the field, caused by this fungus, usually occur just prior to senescence but do affect stand productivity.

The disparity between the numbers of seeds sown and the numbers of seedlings established in each microplot was most likely the result of physical impedance caused by the 10 mm depth of soil above the seeds. Increasing the depth of seed in soil results in reduced seedling emergence of annual medics (Adem 1977; Carter 1987; Carter and Challis

1987). No evidence of phytotoxicity was detected in respect to plant density or morphological features of the top growth of medic seedlings.

3.7 Conclusions

The data collected from the surveys of 12 farm sites quantifies the deleterious effects that cereal straw residues have on regeneration and productivity of annual medic pastures. Although there was considerable variation in plant density from site to site, stubble remaining from the previous crop proved consistently harmful. Results from Field Experiment 1 support this conclusion. Field Survey 2 and Field Experiment 1 provided indirect evidence which suggested that there was marked retardation of breakdown of hard-seededness in annual medic due to thermal insulation by cereal residues on the soil surface. The sites surveyed in this project were selected according to set criteria thus they were not necessarily a representative sample of fields in the districts involved, however, the performances of these regenerating pastures are a good illustration of the possible harmful effects of cereal stubbles.

The types of quantitative, curvilinear relationships derived from the data during these studies, have not been reported previously and these greatly increase our understanding of the importance of cereal stubbles on subsequent pasture productivity. Further experiments are needed to elucidate the relevance of straw particle size distribution and the spatial configuration of stubble i.e. horizontal orientation (often seen after farmers roll stubbles) or vertical orientation of straw and how this may affect temperature gradients in stubble. Upright straw permits greater penetration of thermal radiation and it markedly reduces air turbulence close to the ground thus higher soil temperatures would be expected than if the straw was flattened. Additionally it is desirable to ascertain how variation in summer climatic conditions, especially rainfall and temperature patterns, may modify the response of medic to high straw concentrations.

Barley crops rather than wheat crops appeared to present greater problems for regeneration of annual medics, this is attributed to the physical condition in which stubbles

are left after grain harvest. These surveys provided no information on the outcome which could be expected from less important cereal crops such as oats, rye and triticale, but oats in particular have a growth habit similar to that of barley, therefore comparable effects may occur.

Apart from the effects of straw residues on changes in medic hard-seededness, high concentrations of residue had the capacity to further inhibit seedling emergence, but this occurred erratically and the underlying causes could not be established from the observations made in Field Survey 2.

If pasture productivity is to be optimized and the deleterious effects of straw minimized then there are several farming practices which deserve review. Conclusions drawn from these studies vindicate the advice which has been given in the past i.e. grazing management strategies offer potential for alleviation of the problems posed by heavy straw residues, particularly if sheep or other livestock are introduced to cereal stubble shortly after grain harvest, instead of delaying grazing until mid or late autumn. Stocking intensity may also be manipulated so stubbles are consumed and trampled by livestock more quickly. There may be scope to supplement the low quality roughage which constitute most stubbles with higher protein feed, for example lupin grain, which may enable a higher intake of stubble by livestock (Smith *et al.* 1984; Aitchison *et al.* 1986; Rowe and Ferguson 1986; Coombe *et al.* 1987). Nitrogen supplements (e.g. urea) and energy supplements (e.g. molasses) or chemical modification of the straw (treatment with alkalis) could be considered for evaluation (Mulholland *et al.* 1976; Jackson 1977; Stephenson *et al.* 1984). Straw residues may have a wide range in digestibility and composition which is not well recognized and the factors responsible are poorly understood (Pearce *et al.* 1979, 1986). There have been no studies on the relative rates of ingestion by livestock of current genotypes of cereal straw in Australia. The feasibility of partial removal of stubble may be practicable especially if the portion removed were treated to improve its nutritional value to stock and used as a fodder reserve. Commercial use of straw in manufacturing or for use as an alternative energy source warrants some consideration (McCann and Saddler 1976; Stewart *et al.* 1979; Larson and Turner 1986). In order to minimize the deleterious effects

of straw the quantities present need to be reduced before mid-summer, regardless of the techniques employed.

Alternative strategies exist, new cultivars of medic could be developed with increased rates of breakdown of hard-seededness. The recently released *M. truncatula* cultivar Paraggio was selected because it exhibited accelerated breakdown of hard-seededness (Lake and Crawford 1985) and this feature should be incorporated into a wider range of medics in future. By changing the types of tillage machinery or adjusting the depth of cultivation, seed could be retained closer to the soil surface where the combined effects of seed burial and straw mulch on retarding breakdown of hard-seededness would be reduced. Tillage implements and tillage frequency need to be examined with respect to their influence on legume seed distribution in soil (Taylor 1980, 1985; Quigley *et al.* 1987; Carter *et al.* 1988).

4

THE INFLUENCE OF CROP RESIDUES ON BREAKDOWN OF HARD-SEEDEDNESS IN ANNUAL MEDICS

4 THE INFLUENCE OF CROP RESIDUES ON BREAKDOWN OF HARD-SEEDEDNESS IN ANNUAL MEDICS

4.1 Introduction

Apparent differential changes in hard-seededness caused by a range of straw concentrations were estimated in Field Survey 2 from the emergence of seedlings from moistened soil cores. However, other factors were likely to have influenced the numbers of seedlings besides changes in seed impermeablity. Firstly, the amount of soil above the seed would have impeded emergence. The distribution of seed in the soil profile would be expected to vary depending on previous cultivation practices of farmers and on the soil type: for example, medic pods are trodden into sandy-surfaced soils by livestock but on hard-setting soils, pods remain on the surface. The emergence of annual legume seedlings has been shown to depend on the depth of seed in the soil and on soil texture (Black 1955, 1966; Adem 1977; Carter 1987; Carter and Challis 1987), therefore the emergence figures in Field Survey 2 would have underestimated the amount of permeable seed present in the cores. Secondly, pre-emergence pathogen attack could have reduced seedling density (Andrew 1963; Stovold 1974; Wong *et al.* 1984).

4.2 Field Experiment 3: The influence of cereal stubble and seed depth in soil on breakdown of seed impermeability in annual medics

To evaluate the various factors which contribute to changes in hard-seededness of medics, the following field experiment was initiated to allow more strict control of the seed environment and to make a direct assessment of changes in hard-seed levels after seed was retrieved from the field. A factorial combination of treatments was imposed consisting of seed from three different sources, seed buried at three discrete depths in soil, with three levels of cereal straw residue.

Burton (1964) conducted an experiment at Urania on Yorke Peninsula, where he imposed a range of crop-residue concentrations above resident medic seed reserves. After

several months, but prior to the break in the season, he collected samples of medic seed from soil at three depth intervals and compared the germination percentages. The experiment reported below differed from that of Burton (*loc. cit.*) as seed was placed at discrete depths in soil, three seed sources were compared at The Waite Institute, Urrbrae and the seed was permitted to germinate in the field after the growing season commenced so all normal stimuli to germination were retained.

4.2.1 Materials and methods

Pods and seeds

The first seed source comprised pods from 30 soil-core samples of *Medicago truncatula* cv. Hannaford which were collected from Site 3 (described in Chapter 3) on September 11, 1985 using a Coile sampler. Fifteen samples were taken from soil covered by straw walker rows and fifteen were taken from soil between straw rows. The seed in these pods was considered to be representative of seed which commonly comprises soil reserves. After separating the pods and soil by wet sieving and drying at low temperatures, the pods were hand threshed by gently rubbing them between two sheets of corrugated rubber mounted on wooden blocks. Debris from the pods was removed by sieving and hand sorting. Each seed sample was held at 20^0C in a humidified incubator for seven days to assess germination of soft seed and the percentage of hard seed. The mean germination percentage of this seed and its standard deviation was 9.2±3.1. The residual hard seeds were then dried at ambient temperatures for 12 hours. All hard seed from the 30 samples was bulked and 60 lots of 100 seeds were counted and each lot enclosed in a 70 x 70 mm polyester bag (20207 WTD Marix© thermal screen)†. Small plastic labels (10 x 10 mm) for identification accompanied the seeds.

On March 13, 1984, pods of *Medicago truncatula* cv. Jemalong were collected from the soil surface of a field at Two Wells. The field had been sown and subsequently harvested for the production of commercial medic seed. The recent history of the field and

†Supplied by J.W. Cranston and Co Ltd, 815 Port Rd, Woodville, South Australia

the appearance of the pods indicated that they had all been set in the previous growing season. These medic pods were stored at 20°C until further processing.

In December 1985, 7000 of these pods were separated into 10 batches then moistened with deionized water and stored on wet filter paper at 20°C in order to assess the proportion of soft seed. After three days, germinated seeds were counted and removed then the pods were dried in a forced-draught dehydrator for 20 hours at ambient temperature (~ 18°C). The mean seed germination was 1.4 per cent assuming that there were 6.84 seeds per pod (see below for measurement of seed per pod). Next, 5800 of these pods were divided into batches of 100 and each batch enclosed in a polyester bag. These unthreshed Jemalong seeds in pods comprised the second seed source. The remaining 1200 pods were then rubbed between corrugated rubber sheets and the cleaned seed counted into 70 lots of 100. A further germination test on this seed was made over seven days by moistening the seeds in petri dishes and maintaining them at 20°C. Abrasion of the residual hard seeds in the pods during the extraction process resulted in a germination percentage 14.4± 1.8 and the mean number of seeds per pod was 6.84±0.26. Germinated seeds in 60 of the lots were replaced with ungerminated seeds from the remaining 10 to give a total of 100 hard seeds in each of the 60 lots. Each lot was air dried and enclosed in polyester bag: this group of Jemalong seeds comprised the third seed source.

Additionally, 2000 Jemalong pods from Two Wells were separated into batches of 100 and weighed. Ten batches were moistened in petri dishes, held at 20°C and seed germination counted after seven days. This germination percentage was determined to be 1.4±0.3. After drying at ambient temperature the remaining seeds were threshed out and seeds counted and weighed. The relationship between pod weight and seed numbers within the pods was determined by linear regression methods. The derived equation was:

$$Y=304.1+54.3X \qquad r^2=0.60\ **$$

where Y is the seed number in 100 pods and X is the weight of 100 pods (g). From this relationship, the number of Jemalong seeds in each polyester bag (mentioned above) were estimated based on the weight of pods in the bag.

The remaining ten lots of 100 pods, together with spare samples of Hannaford and Jemalong seed were stored at 20°C as controls and processed at the conclusion of the field experiment when germination percentages were assessed.

Site details

The field experiment was located on three consecutive plots of wheat stubble (the same genotype), each 4 m x 0.75 m (see Figure 4.1), and on the pathways between the plots, 1.6 m x 0.75 m. Grain had been harvested on December 1985, using a small-plot harvesting machine. Three ground-cover treatments were imposed, viz. (A) no cover, (original pathways), (B) light cover, (chaff and loose straw removed), (C) heavy cover, (all straw and chaff retained). Each of the original plots was halved and treatments (B) and (C) randomly allocated to four of the new plots giving two replicates and leaving two spare areas. Stubble density was measured by collecting all above-ground residues from ten 40 x 40 cm quadrats placed at random on the two spare areas. All chaff and straw removed from treatment (B) was collected. The collected residues and quadrat samples were dried in a forced-draught dehydrator at 80°C for 24 hours and then weighed.

The polyester bags containing seeds or pods were placed between the wheat straw rows (15 cm apart). Three bags representing each seed source were placed at each depth: 2.0 cm, 1.0 cm or on the surface, in each of the ground-cover plots on January 21, 1985. A schematic representation of Plot 1 is shown in Figure 4.2 to indicate the layout of treatments. Bags on the soil surface were anchored by driving 35 mm long, flat-head nails through the corners of the bags into the soil. All bags were retrieved from the soil on May 15, 1986 after 114 days in the field. Photographs of three treatment plots are provided in Plate 4.1 to indicate the visual differences in straw densities and the placement of seed bags.

Seed analysis

Seeds or pods from each bag were counted and in the case of seeds the difference between the number originally present and the number retrieved was assumed to have germinated. Residual seeds and pods were transferred to petri dishes containing two

Whatman, qualitative No.1 filter papers and seeds were then moistened with 5 ml of 0.128% (W/V) Thiram (fungicide) in deionized water; pods were moistened with 15 ml of the same solution. All dishes were placed in a humidified incubator at 20°C and germination was assessed at two or three day intervals for 10 days. After this period lids were removed from the dishes and the contents allowed to dry at 20°C for 24 hours. Dishes were transferred to an incubator which maintained temperature at 50°C for 7 hours and 15°C for 13 hours each day; the remaining time was at transitional temperatures. After 10 days the dishes were moistened with deionized water and held at 20°C and germination was measured for 10 days. The alternating temperature treatment followed by 10 days at 20°C was repeated. The ten lots of pods not used in the field experiment underwent the same germination assessment as described above for pods from the field experiment.

After these germinations the pods were air dried and threshed in a Wiley mill fitted with composite rubber beaters instead of steel beaters, after which debris was removed by aspiration and the seeds counted.

Treatment effects were statistically evaluated by analysis of variance.

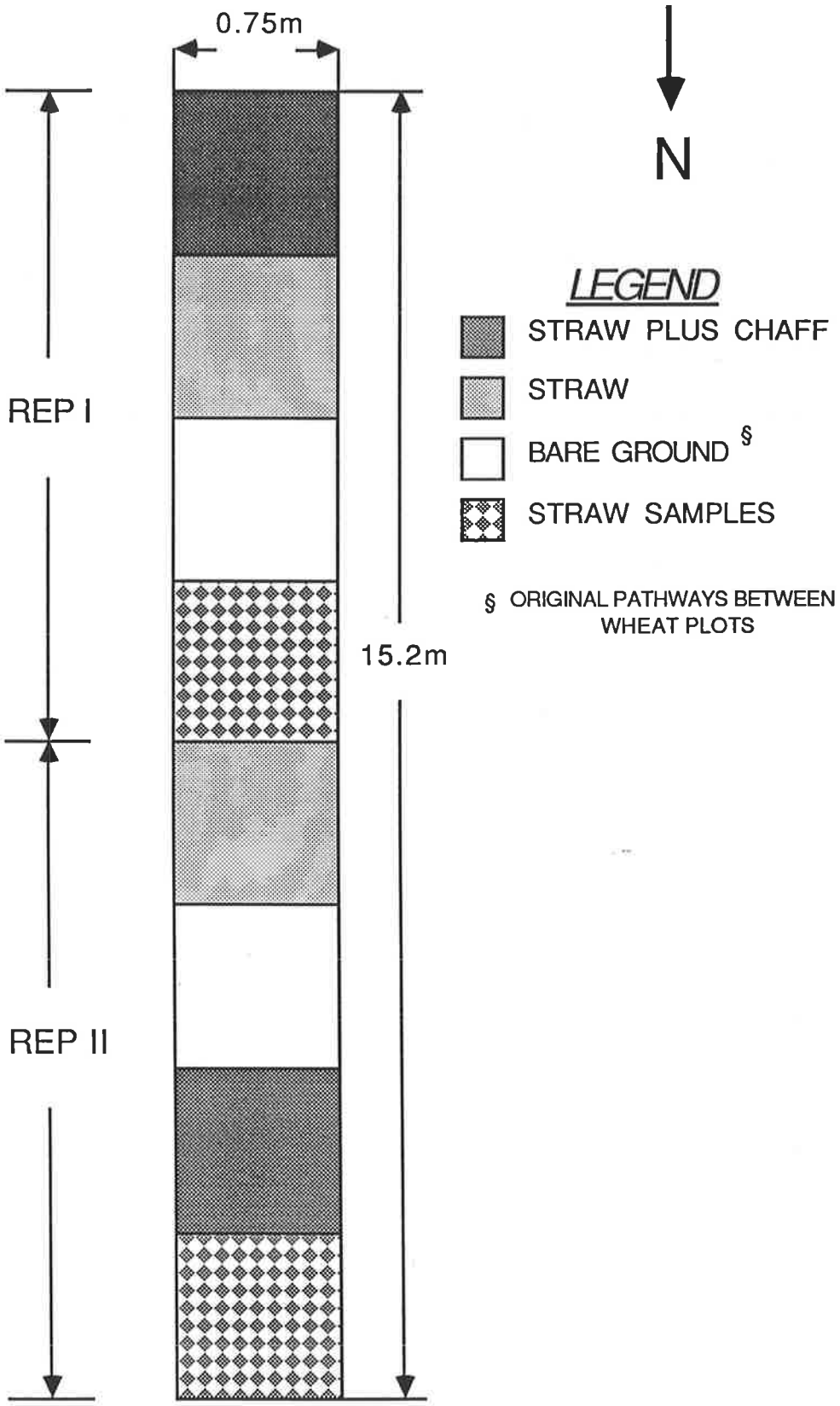


FIGURE 4.1: Schematic diagram of the experimental area at The Waite Institute, Urrbrae used for Field Experiment 3

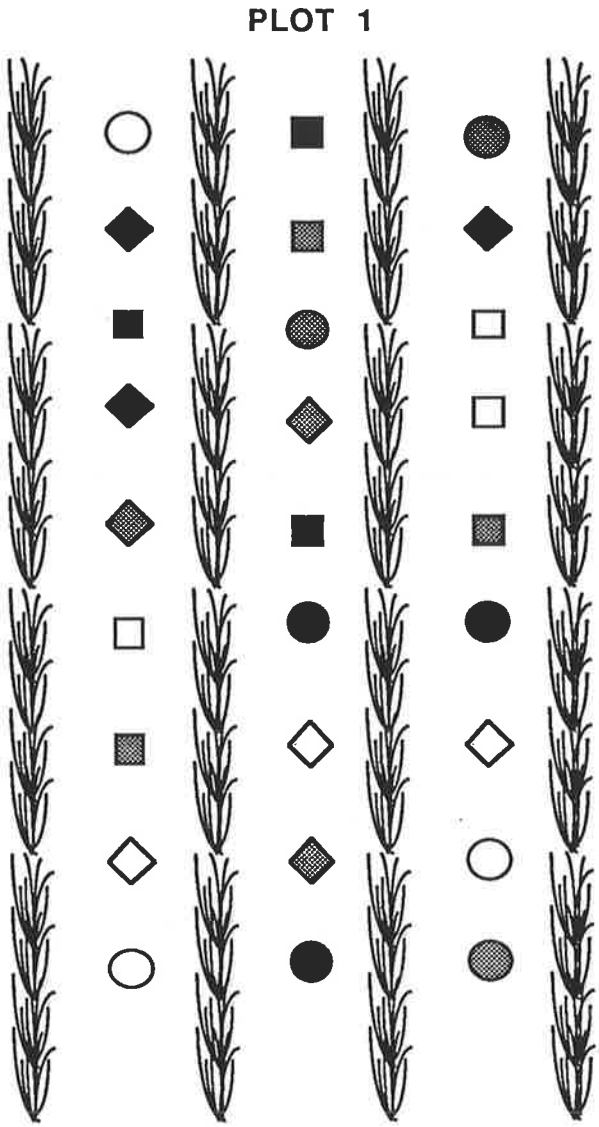


FIGURE 4.2: Schematic representation of Plot 1 indicating the arrangement of treatments relative to existing rows of wheat stubble.

Key to symbols

Seed source	**Depth**
Squares - Hannaford seed	White - on the soil surface
Circles - Jemalong seed	Grey - 1 cm deep
Diamonds - Jemalong pods	Black - 2 cm deep

PLATE 4.1: Field Experiment 3 - general views of three plots with different covers of straw.

A No straw

B Light cover of standing straw (3254 kg/ha), similar to that often found between walker rows on farms

C Heavy straw concentration (6711 kg/ha), much like the densities found in straw walker rows


A
B
C

4.2.2 Results

The mean concentration of cereal residue originally on the plots (treatment C) was 6711±1129 kg/ha and the mean concentration of straw and chaff removed from treatment B i.e. light cover, was 3254±995 kg/ha leaving a straw concentration in this treatment of 3475 kg/ha.

Germination of seed in the field

All experimental factors viz. stubble concentration, seed depth in soil and seed source were responsible for significant differences in the numbers of hard seeds retrieved from the field. Treatment means are provided in Tables 4.1 and 4.2. The seed which was not retrieved was presumed to have become permeable and germinated or the soft seed decayed by microbial attack after imbibition. These differences in residual hard-seed levels were caused by variation in breakdown of hard-seededness during the period in the field.

Analysis of variance for germination percentage of seed in the field

Source of variation	DF	Sum of squares	Mean square	F value	Significance level
Reps	1	158.57	158.57		
Ground cover	2	1088.51	544.26	16.997	*
Residual	2	64.04	32.02		
Total	4	1152.56	288.14		
Seed source	2	1205.31	602.66	23.616	**
Depth	2	723.93	361.96	14.184	**
Seed source x Depth	4	581.40	145.35	5.696	**
Ground cov. x Seed source	4	185.05	46.26	1.813	ns
Ground cov. x Depth	4	136.01	34.00	1.332	ns
Ground cov. x Dep. x Seed	8	286.52	35.81	1.403	ns
Residual	125	3189.90	25.52		
Total	149	6308.12	42.34		
Grand total	154	7619.24			

Germination percentages of seed in each ground-cover treatment are presented graphically in Figure 4.3. Increasing quantities of cereal residue appeared to have least influence on breakdown of hard seed in pods of Jemalong medic. Apparently the thermal insulation afforded by the pods moderated the diurnal temperature fluctuations experienced by the seeds within the pods, consequently there was less stimulus for change in seed coat permeability. On the bare soil treatment about 15 per cent of the seeds in pods became

permeable over summer and autumn which indicates that in farm situations reserves of seed may persist for lengthy periods provided pasture and soil management is appropriate for seed survival. Data which substantiates this claim has been obtained by Carter (1985) who has recorded cumulative emergence of medic seedlings from buried pods and sheep faecal pellets for 12 growing seasons. During this period plants were removed to prevent fresh seed production.

The influence that increasing depth of burial has on the proportion of permeable seed, in late autumn, is shown in Figure 4.4. Analysis of variance indicated that there was a significant interaction between seed source and seed depth in soil. This was the result of a high germination percentage of Hannaford medic seed after burial at 1.0 cm depth in the soil. This was unexpected as germination in this particular treatment exceeded the germination percentage of Hannaford seed at the soil surface. The pattern set by Hannaford seed at the three depths was a departure from the trend exhibited by Jemalong seed in pods and free Jemalong seed where germination decreased with increasing depth in the soil. Moreover, the Hannaford germination at 1.0 cm would not be expected if soil temperature was the major determinant of changes in seed coat impermeability whereas the pattern set by the Jemalong treatments support the assertion that increasing depth of seed in the soil impairs breakdown of hard-seededness because of moderation of temperature fluctuations in the soil.

The mean germination of Jemalong seed in pods was 13.3 per cent and the corresponding figure for seed out of pods was 18.8, a difference of 5.5 per cent. Similar ranges in germination percentages were found between the extremes of straw cover (18.3 - 13.0) and extremes of seed depth in the soil (19.9 - 13.9), when pooled germination values for Jemalong seed in pods and seed free of pods were compared. The relative reduction in germination percentage in each of these cases exceeds 25 per cent. The data from this experiment also suggest that the effects on seed coat permeability by straw cover and seed burial in soil are additive.

Germination of residual seed in the laboratory

The mean germination percentage of all seed retrieved from the field experiment then held at 20°C for ten days was only 0.43 per cent. Differences between treatments were too small to be of consequence.

After treatment with alternating temperatures ($50^0/15^0$C for 10 days) further germination of 3.8 per cent was recorded and after a second period of these temperatures an additional germination of 4.3 per cent occurred. Analysis of variance of the data (transformed $\log_e x+1$) from each treatment indicated that seed source, depth in soil and mulch concentration all had significant effects on germination percentages.

Analysis of variance for germination percentage of seed in the laboratory

Source of variation	DF	Sum of squares	Mean square	F value	Significance level
Time	1	7.27	7.27	6.168	ns
Residual	2	2.36	1.18		
Total	3	9.62	3.21		
Ground cover	2	19.95	9.98	254.364	**
Time x Ground cov.	2	2.06	1.03	26.283	**
Residual	4	0.16	0.04		
Total	8	22.17	2.77		
Seed source	2	101.10	50.55	205.695	**
Depth	2	6.47	3.24	13.173	**
Time x Seed source	2	2.74	1.37	5.565	**
Seed source x Depth	4	3.89	0.97	3.952	*
Ground cov. x Seed source	4	9.76	2.44	9.925	**
Time x Depth	2	1.57	0.79	3.195	*
Ground cov. x Depth	4	1.88	0.47	1.907	ns
Time x Ground cov. x Seed	4	6.39	1.60	6.502	**
Time x Gr. cov. x Depth	4	0.09	0.02	0.094	ns
Time x Seed x Depth	4	1.26	0.32	1.284	ns
Ground cov. x Dep. x Seed	8	3.18	0.40	1.619	ns
Residual	262	64.39	0.25		
Total	302	202.71	0.67		
Grand total	313	234.50			

There was no significant difference between the results of the two germinations (designated "Time" in the analysis). The sum of the three germinations, determined in the laboratory, are given in Tables 4.3 and 4.4. Most noteworthy, the trends for each treatment in these germinations were the reverse of the trends shown in the field.

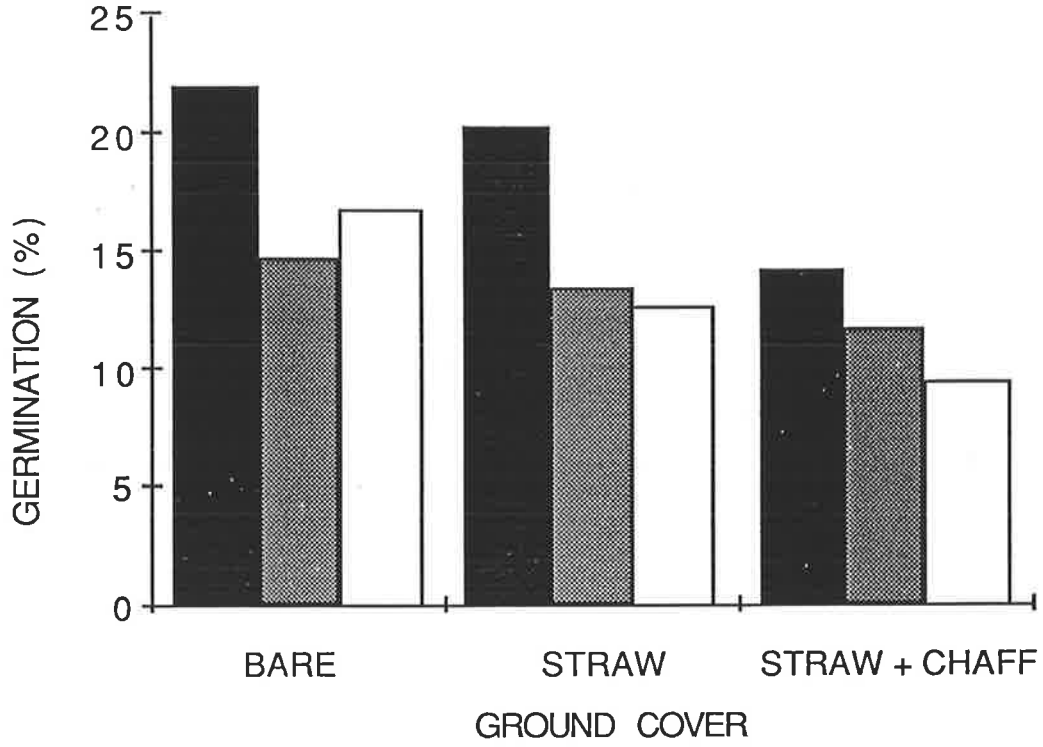


FIGURE 4.3: The influence of a range of ground covers on the germination percentage of three seed sources of *M. truncatula* seed in the field.

Legend

- Jemalong seed free of pods
- Jemalong seed in pods
- Hannaford seed free of pods

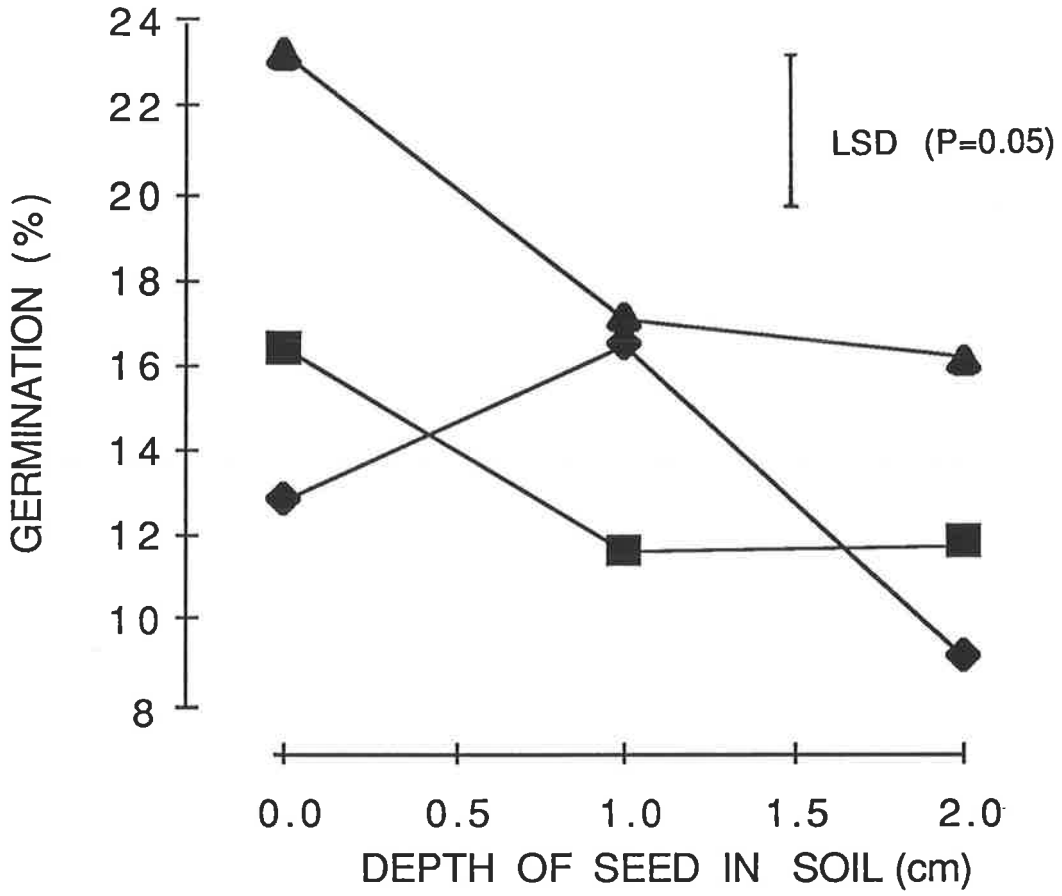


FIGURE 4.4: The influence of depth of seed in soil on the changes in permeability of *M. truncatula* seed from three sources.

Legend

■ Jemalong seed in pods

▲ Jemalong seed free of pods

◆ Hannaford seed free of pods

TABLE 4.1: Field Experiment 3 - mean seed germination percentages in the field, for single treatments and combinations of treatments.

		Seed source		
		Hannaford seed	Jemalong seed	Jemalong pods
		12.8	18.8	13.3
		Seed depth (cm)		
		0	1	2
		17.5	15.1	12.3
		Ground cover		
		Chaff+Straw	Straw	Bare
		11.6	15.5	17.9
		Seed depth (cm)		
		0	1	2
Seed source	Hannaford seed	12.8	16.5	9.1
	Jemalong seed	23.3	17.1	16.1
	Jemalong pods	16.4	11.7	11.8
		Seed source		
		Hannaford seed	Jemalong seed	Jemalong pods
Ground cover	Chaff+Straw	8.6	14.3	11.8
	Straw	12.8	20.2	13.4
	Bare	16.9	21.9	14.7
		Seed depth (cm)		
		0	1	2
Ground cover	Chaff+Straw	15.8	11.0	8.0
	Straw	17.6	15.3	13.6
	Bare	19.2	18.9	15.5

TABLE 4.2: Field Experiment 3 - mean seed germination percentages in the field, for all treatments combinations.

Ground cover	Seed source: Hannaford seed			Seed source: Jemalong (loose seed)			Seed source: Jemalong (seed in pods)		
	Depth of seed in soil (cm)								
	0	1	2	0	1	2	0	1	2
Chaff+Straw	8.2	12.0	5.7	23.4	10.5	9.0	15.8	10.3	9.2
Straw	12.5	16.2	9.8	24.2	17.5	19.0	16.0	12.3	11.9
Bare	17.7	21.2	11.8	22.4	23.2	20.3	17.5	12.4	14.3

TABLE 4.3: Field Experiment 3 - mean seed germination percentages in the laboratory, for single treatments and combinations of treatments.

(Seed held at 50/15^{0}C for a total duration of 20 days)

Seed source		
Hannaford seed	Jemalong seed	Jemalong pods
1.0	1.7	6.5

Seed depth (cm)		
0	1	2
2.0	2.4	3.1

Ground cover		
Chaff+Straw	Straw	Bare
3.8	2.3	1.6

	Seed depth (cm)		
	0	1	2
Seed source			
Hannaford seed	1.1	0.8	1.2
Jemalong seed	1.1	1.8	2.4
Jemalong pods	4.6	6.9	8.5

	Seed source		
	Hannaford seed	Jemalong seed	Jemalong pods
Ground cover			
Chaff+Straw	1.1	3.6	10.4
Straw	1.1	1.1	6.8
Bare	0.9	1.1	3.7

	Seed depth (cm)		
	0	1	2
Ground cover			
Chaff+Straw	2.5	4.2	5.1
Straw	2.0	2.1	2.8
Bare	1.4	1.4	2.1

TABLE 4.4: Field Experiment 3 - mean seed germination percentages in the laboratory, for all treatments combinations.

(Seed held at 50/15^{0}C for a total duration of 20 days)

	Seed source								
	Hannaford seed			Jemalong (loose seed)			Jemalong (seed in pods)		
	Depth of seed in soil (cm)								
Ground cover	0	1	2	0	1	2	0	1	2
Chaff+Straw	0.9	0.9	1.5	2.7	3.9	4.3	5.0	13.4	16.0
Straw	1.4	1.0	1.0	0.6	1.2	1.6	6.1	5.7	9.0
Bare	1.1	0.4	1.1	0.6	1.0	1.8	3.1	4.0	4.0

4.2.3 Discussion

In this experiment, both burial of seed in soil, and mulches of cereal residue, retarded breakdown of hard-seededness of *M. truncatula* in the field: similar magnitudes of retardation occurred for each factor.

For seed of cv. Jemalong, in intact pods, the highest germination in the field was 17.5 per cent with no cover over the seed while the lowest germination was 9.2 per cent with deep cover by soil and mulch. This range represents almost a two fold difference, however, seed not insulated by pods exhibited even wider variation than this, especially cv. Hannaford. In paddocks, seed may not necessarily remain in pods as physical degradation of pods proceeds with ageing and following soil tillage: these factors would influence the extent to which pods may moderate temperature fluctuations experienced by seed.

The seed population of cv. Hannaford consisted of seeds of different ages and these would have been exposed to different ranges of temperatures in the field prior to the original collection of seed from the field at Mallala. It is important in the future to establish whether there are major differences in the patterns of breakdown of hard-seededness in freshly formed seed compared with aged seed reserves in the field and whether there are differential responses to variations in thermal stimuli.

Seed germination percentages of cvv. Hannaford and Jemalong were markedly different but it is not possible from this experiment to conclude that the major cause of the difference is genetic as the origins and histories of these seeds differed. Seedlots of the same genotype of *M. truncatula*, grown under different environmental conditions have been shown to have dissimilar patterns of hard seed breakdown (Bolland 1986) and maturation conditions of seed and cultural practices may have a major impact on the subsequent softening of seed of subterranean clover (Quinlivan 1965a; Collins 1978, 1981) : however, the interrelationship between environment and seed responses are not well understood (Taylor 1984).

Genetic influences on breakdown of hard-seededness have been investigated in the past. Quinlivan (1968a) showed different rates of seed softening in four species of *Medicago* and Hagon (1974) was able to differentiate between patterns of breakdown of

hard-seededness in four cultivars of *Medicago truncatula*: e.g. cv. Jemalong had a markedly higher proportion of hard seed than cv. Hannaford during early autumn, thus breakdown of hard-seededness in Hannaford is more sensitive to temperature fluctuations.

The reversal of the trends in seed germination during assessment of hard-seededness in the laboratory, compared with patterns in the field, (as shown in this thesis study) is the first observation reported for *Medicago* although similar reversal has been described for seed of subterranean clover (Taylor 1981, 1984). This stimulation of germination of residual hard seeds following additional thermal stimulus may, in the long term, cause levels of hard-seededness of a particular cultivar to be more homogeneous in any given field situation. This matter ought to receive additional research attention as it is important to know the variability of potential germination of seed reserves in paddocks if we are to optimize medic plant densities.

The data on medic seed germination obtained in this field experiment at the Waite Institute, ranging between 24.2 per cent and 5.7 per cent, is similar to the germination data (range 25.0 to 6.2 per cent) obtained by Burton (1964).

4.3 Field Survey 4: The influence of cereal stubble and seed depth in soil on the incidence of seed impermeability in annual medics

In the previous experiment, increasing depth of burial of medic seed was associated with lower proportions of seed which were permeable in autumn. Heavy concentrations of cereal residue also retarded breakdown of hard seed over summer. It was desirable to substantiate this variation by examining the proportion of hard seed in paddocks, differentiating between seed† on the soil surface and seed† in the top 5 cm of soil, as well as comparing effects of straw in walker rows and straw between walker rows. To meet these objectives, seed samples were gathered from selected sites in order to determine the levels of hard-seededness in paddocks under typical farmer management. Seed permeability was measured directly by separating the seed† from soil before undertaking germination tests.

4.3.1 Materials and Methods

Collection in the field

The three sites in this investigation were all barley stubbles. Site 1 was located 12 km west of Mallala on the Adelaide Plains. On May 2, 1986 straw samples were collected from ten 40 x 40 cm quadrats located on straw walker rows and from ten quadrats located between walker rows. Barrel medic pods on the soil surface were also collected from the quadrats. Five soil cores (5 cm deep) were taken from the area within these same quadrats using a Coile sampler and each group of five cores were bulked. In addition approximately 250 pods were collected at random from beneath walker rows and 250 pods collected from between walker rows (non-quadrat areas).

Site 2, located at Redbanks on the Adelaide Plains, was sampled on May 8, 1986 using the same methods as for Site 1.

Pods from beneath straw walker rows and from between rows were also collected from Site 3 at Redbanks the same day, but no soil samples were taken.

† Most of the medic seed was in pods

Laboratory analyses

Surface samples

For Site 1, quadrat samples of pods collected from the soil surface were counted and weighed to ascertain their density in the paddock. The pods not originating from the quadrats were also counted and weighed then divided into batches of approximately 50. Each group of pods was placed in a petri dish with two Whatman qualitative No.1 filter papers and moistened with 15 ml of 0.128% (W/V) solution of Thiram in deionized water. Dishes were maintained at 20°C in a humidified incubator and germinated seeds were counted and removed over the next 21 days. Moisture was maintained in the dishes by adding deionized water during this period. Lids were then removed from all dishes and the pods allowed to dry in a laboratory where temperature was held at 20°C. The dishes were then transferred to an incubator which maintained 50°C for 7 hours and 15°C for 13 hours each day (with the remaining four hours at transitional temperatures), similar to the temperatures experienced at the soil surface over summer and autumn. After ten days the contents of the dishes were moistened with 15 ml of deionized water and returned to the original incubator at 20°C for further assessment of germination over the next 7 days. Finally after the pods were dried and threshed, the seeds were sorted from the debris and then counted and weighed. Pods collected from quadrats were also threshed and the seed number and weight measured.

Pods from quadrat samples at Site 2 were counted but because of low pod densities germination was not assessed on this material. Those pods not collected from quadrats were divided into groups of 25 and germinated in the same manner as for Site 1. Similar germination techniques were used for pod samples from Site 3.

Straw samples were dried in a forced-draught dehydrator at 80°C for 24 hours then weighed.

Soil samples

Soil samples from Sites 1 and 2 were soaked in tap water for 20 minutes and gently agitated on a 0.7 mm square-mesh sieve in tap water to remove most of the soil. Gravel and organic matter remaining in the sieve was washed into a large plastic beaker partly filled with tap water The floating pods and other organic matter were transferred to a 0.7

mm square-mesh sieve, drained then dried for 12 hours at ambient temperature in a forced-draught dehydrator. Pods were separated from the residue by hand. Pod samples from each quadrat at Site 1 were placed in a petri dish: however, because of higher pod densities in soil at Site 2 these pod samples were split into three sub-samples and each sub-sample transferred to a petri dish. A small quantity of loose seed was retrieved in samples from Site 1 but not from Site 2, these were retained with the pod samples. All pod samples were germinated using the same techniques as described above for surface samples after which seed was extracted from the pods then counted and weighed.

Comparisons of mean germination percentages for seed from beneath walker rows and for seed from between walker rows were made using Student's *t*-test. The relationship between percentage germination of seed collected from beneath the straw (seed on the soil surface) and straw concentration was evaluated for Site 2 using regression techniques.

4.3.2 Results

The seed reserves from pods on the soil surface were of similar magnitude at both Site 1 and Site 2 (Table 4.5) but the quantities of seed within the top 5 cm of soil (excluding the surface) were greater at Site 2. The low numbers of seed per pod at both sites suggests that the pods were comparatively old i.e. a high proportion of the original seed in the pods had become permeable and germinated in previous years.

High concentrations of straw in the walker rows impeded the breakdown of hard-seededness and the seeds in pods on the soil surface were affected most by the presence of dense straw residues (Table 4.6). At all three sites there was a significantly higher proportion of permeable seed between the walker rows compared with seed beneath walker rows; differences in germination percentage exceeded two fold. The same trend in seed germination appeared in the case of buried pods in the soil but effects of straw concentration were not as marked and differences were not statistically significant.

The potential seedling densities can be calculated as the product of the seed/unit area and the germination percentage of the seed (Table 4.6). However, in this simple derivation, there is an assumption that deep layers of straw in the walker rows do not affect the emergence of seedlings and their subsequent establishment; this assumption will be

discussed in detail in Chapter 6. The large differences in germination percentages of seed on the soil surface were responsible for large relative differences in potential seedling density. About three times as many seedlings could be expected from seed, on the soil surface, between walker rows compared with seedling density within walker rows. If all the seed at Site 1 had been on the surface then approximately 150 medic plants/m^2 could have been expected between the walker rows rather than 68 plants/m^2. Similarly at Site 2, if all seed had been on the soil surface then medic density between the rows would have increased from 62 plants/m^2 to approximately 320 plants/m^2. These changes illustrate the considerable improvements in medic density possible by retaining seed closer to the soil surface.

The relationship between straw concentration and seed germination at Site 2 was not statistically significant when a first order polynomial model was tested by linear regression: however, a significant relationship existed when data was fitted to an exponential model and the equation derived was -

$$Y=16.06e^{-0.00016X} \quad r^2=0.247 * \quad (n=20)$$

where Y is germination percentage of seed and X is straw concentration (kg/ha).

The alternating temperature treatment, imposed on the seeds in the laboratory for 10 days, stimulated little change in seed-coat permeability but this time of exposure was relatively brief. After treatment, the seed germination percentages ranged between 0.4 - 1.4 per cent and it was not possible to draw any conclusions about the effects of straw mulches on subsequent breakdown of hard-seededness.

TABLE 4.5: Pod and seed densities of *Medicago truncatula* on the soil surface and within the top 5 cm of soil, at two field sites on the Adelaide Plains, 1986.

Site	Straw concentration (kg/ha)	Pod reserve (kg/ha)	Pod reserve (#/m^2)	Seed reserve (kg/ha)	Seed reserve (#/m^2)	Seed per pod
Site 1						
(a) Soil surface						
Walker row	5229	55	172	3.6	143	0.97
Between	1664	59	169	4.9	188	1.35
(b) Top 5 cm soil (excluding surface)						
Walker row	-	589	1301	34	1289	1.05
Between	-	479	1226	26	1026	0.88
Site 2						
(a) Soil surface						
Walker row	3078	60	97	3.2	103	1.05
Between	1288	96	164	4.2	131	0.78
(b) Top 5 cm soil (excluding surface)						
Walker row	-	1498	3344	73	2327	0.70
Between	-	1540	3408	63	2000	0.59

TABLE 4.6: Seed permeability and potential seedling densities of *Medicago truncatula* as influenced by heavy concentrations of barley straw residue in walker rows.

Site		Seed germination (%)		Potential seedling density (#/m^2)	
		Seed from soil surface	Seed from 0-5cm soil†	Seed from soil surface	Seed from 0-5cm soil†
Site 1- Mallala	Walker row	5.8	3.8	8.3	49.0
	Between	12.7	4.3	23.8	44.1
		**	ns		
Site 2- Redbanks	Walker row	5.6	1.5	5.8	34.9
	Between	14.9	2.1	19.5	42.0
		**	ns		
Site 3- Redbanks	Walker row	10.2	-	-	-
	Between	22.0	-	-	-
		**			

† Excluding soil surface

** $P < 0.01$; ns, not significant

4.3.3 Discussion

Although the number of paddocks surveyed was a limitation of this study, the data substantiates the results found in the previous experiment (Field Experiment 3: The influence of crop residues on breakdown of hard-seededness of annual medics). Medic seeds on the soil surface were particularly sensitive to thermal insulation by cereal residues so breakdown of hard-seededness was inhibited by high concentrations of straw residues. These same trends were shown by seed reserves beneath the soil surface but in this category of seed the overall changes in hard-seededness were small and differences were not significant. These results clearly indicate that medic seed distribution in soil profiles can have a major bearing on potential seedling densities in paddocks. This data vindicates the previous claims made that seed reserves in paddocks ought to be maintained near the surface (Carter 1981, 1982, Carter *et al.* 1982).

Preliminary data has been collected describing the profile distribution of medic seed in soil cores collected from farm paddocks (Quigley *et al.* 1987). These sites were believed to be representative of common situations on farms. Of the three sites examined, two had over 50 per cent of the seed located at soil depths exceeding 2.5 cm, and slightly less than 50 percent of the seed was below 5.0 cm at one of these sites. The distribution of seed in soil depends on - a) the type of tillage machinery used, b) the depth of tillage, and c) soil texture. The influence of cultivation implements on medics has been clearly demonstrated in South Africa in a zone which is climatically and edaphically similar to the wheat zone of South Australia (Table 4.7). This work showed that shallow cultivation enabled higher densities of seedling emergence and ensured that satisfactory seed reserves remained in the top soil.

TABLE 4.7: Effects of tillage in 1986 on medic in 1987, Langgewens Experiment Station, Cape Province, South Africa.

	Mouldboard ploughed 1986	Scarified 1986
March 1987 Medic total seed reserve (kg/ha)		
0 - 5 cm soil	63	450
April 1987 Medic emergence (plants/m^2)	178	1173
June 1987 Medic hard seed reserves (kg/ha)		
0 - 5 cm soil	32	310
5 - 15 cm soil	172	31
> 15 cm soil	7	3

Source: E.D. Carter, P. Thomas, E. Fletcher and E. Cotze, Biennial Report of the Waite Agricultural Research Institute for 1986 and 1987, The University of Adelaide. (In press).

4.4 Conclusions

Results from both the experiment and the survey show how increasing densities of ground cover by cereal residues can directly reduce the breakdown of hard-seededness in annual medics: similarly, changes in seed permeability are retarded as seed is buried deeper in soil. These effects have been noted previously by other researchers, especially with subterranean clover, and were expected here. However, this data illustrates the magnitude of these effects on medics over a number of sites, information hitherto not available. The impact on the rates of seed softening was substantial and of considerable practical significance.

These results are clear evidence of the need to maintain a high proportion of the seed reserve near the soil surface in order to maximize the rate of breakdown of hard-seededness. To achieve this objective, attention needs to be directed towards definition of suitable tillage techniques including frequency of cultivation. Additionally, judicious grazing management is required to minimize the consumption by sheep of medic pods on the soil surface. Large quantities of medic pods can be ingested by sheep but only a low proportion of viable seeds pass through the alimentary tract (Vercoe and Pearce 1960; Denny *et al.* 1979; Carter 1980, 1981). Carter (1981) suggested that shallow burial of medic pods is essential to prevent overgrazing of pods and ensure good regeneration.

The techniques used to study changes in hard-seededness in the field, including emergence of seedlings in intact cores (Chapter 3), burial of seed in pouches (section 4.3) and separation of seed from soil before germination tests (section 4.3) were all useful methods and have potential for application in future research on seed behavior in the field. There is a need to examine the changes in hard-seededness at depths in the soil exceeding 2.0 cm, the level of success seedlings have in emerging from such depths and the influence that pathogens have on these seedlings. Results from section 4.3 indicate that some 20 per cent of seed at 2.0 cm depth in the soil may become permeable over the summer, which is a rather large proportion of the seed reserve. The deleterious effects on seedling emergence caused by burying seed at increasing depth (maximum 3.5 cm deep) are examined in Chapter 6.

5

STUDIES ON PHYTOTOXINS LIBERATED FROM STRAW, USING ANNUAL MEDIC BIOASSAYS

5 STUDIES ON PHYTOTOXINS LIBERATED FROM STRAW, USING ANNUAL MEDIC BIOASSAYS

5.1 Introduction

The object in this chapter is to assess the reactions of annual medic seedlings to crude aqueous extracts from cereal straw. Chemical isolation and identification of phytotoxins was not attempted in these studies as agronomic aspects were accorded higher priority and chemical composition of leachates has been demonstrated to be dynamic (Blum *et al.* 1984). Phytotoxicity may result from compounds released directly from unweathered crop residue or may develop during microbial decomposition of residues (Guenzi and McCalla 1962; Norstadt and McCalla 1963; McCalla and Haskins 1964; Patrick and Toussoun 1965; Toussoun *et al.* 1968). The greatest hazards of phytotoxicity are associated with the early stages of straw decomposition or with unweathered residues (Harris and Kimber 1983). In view of this and the complexity inherent during the decomposition of straw (Patrick and Toussoun 1965; Cochran *et al.* 1977) it was decided that the following studies should concentrate on investigation of leachates from undecomposed cereal residues.

In the ley farming system, regeneration of annual legumes occurs in crop stubbles especially where short rotations are used i.e. commonly in the sheep-cereal production zone (Webber *et al.* 1977), therefore there is frequent opportunity for allelopathic effects to be manifested. It is surprising that potential phytotoxicity arising from cereal crop residues has not been previously investigated in detail, with an annual legume species as the test plant, especially in view of the numerous reports on responses of weed and crop species to phytotoxins liberated from unweathered or decaying crop residues; see reviews by Horsley (1977), Putnam (1978, 1985), Rice (1979, 1984), Harris and Kimber (1983) and Hartley and Whitehead (1985) .

Previous investigations with pasture legumes include experiments by Waddington (1978) who incorporated ground wheat straw into soil and found reduced emergence of alfalfa but that subsequent growth of plants was unaffected by this soil treatment. He

speculated that alfalfa may be especially susceptible to phytotoxins liberated by decaying residues because water enters the seed near the embryo, therefore any toxin may be concentrated in this vulnerable portion of the seed. Similar arguments could apply to annual medics as their seed structure is similar to that of alfalfa seed.

Iswaran and Harris (1968) showed that lucerne cv. Hunter River and subterranean clover cv. Yarloop were more sensitive to toxicity than oat seedlings when wheat straw was incorporated into a coarse, river-sand. Dry matter production was reduced and roots were discoloured and exhibited numerous necrotic lesions but there were no reported effects of straw on legume seedling density. They concluded that initial anaerobic decomposition of straw proceeds when it is buried in soil, largely as a result of bacterial fermentation. They found that the germination of subterranean clover cv. Mt Barker and lucerne cv. Hunter River was markedly retarded in petri dishes which contained filtrates from microorganisms isolated from decaying straw. Furthermore, the elongation of lucerne radicles was inhibited when cultures of the bacteria were present in sand which suggested that secretions from these organisms was responsible. Kloot and Boyce (1982) found that aqueous extracts from mature plants of wireweed (*Polygonum aviculare*) were capable of inhibiting germination of seed of *Medicago truncatula* cv. Jemalong. Cold water extracts from undecayed straw residues of the annual pasture grass *Bromus mollis* have been shown to retard germination of *Trifolium* and *Medicago* spp. (Greenwood and Kimber 1967). Cereal crop residues caused inhibited root elongation of wheat seedlings assessed in a soil-free bioassay (Lovett and Jessop 1982). The phytotoxicity of residues was in the order barley > wheat > oats. However, when residues were allowed to decay for 48 hours these deleterious effects often disappeared.

A wide diversity of techniques have been used to detect inhibitors of seed germination or plant growth and development, and examples of various bioassays were cited by Putnam and Duke (1978) in their review of allelopathy in agroecosystems.

The most commonly-used method for obtaining potential phytotoxins is cold water extraction through simply soaking either dried or green plant parts. These methods are designed to simulate natural release of compounds in the field. After extraction of the material for varying lengths of time, the aqueous solution is usually filtered or centrifuged

to remove solids before bioassaying by petri dish techniques, or in soil or nutrient solution (Putnam and Duke 1978). Frequently, bioassays detect physiological activity of allelochemicals at concentrations much lower than the sensitivity of chemical tests (Leather and Einhellig 1985), hence their utility. The type of bioassay selected may depend on the developmental stage of the plant, the particular aspect of its metabolism being investigated, the quantity of phytotoxin available and the degree to which it has been purified. The development of a bioassay system for study of phytotoxicity was an essential step in these studies as it was necessary to reduce the number of variables which influenced medic seedling ontogeny; assessment in the field was not practicable at this level of investigation.

The eight related experiments reported in this chapter examined reactions of commonly-cultivated annual medics to leachates from cereal straw, variation in effects caused by leachates derived from a range of cereal species and cultivars, together with factors which could possibly have altered the potency of phytotoxins, such as repeated leaching of straw, weathering of residues in the field and phytotoxin contact with soil. These were selected for examination because they represent the most common combinations likely to be encountered in the present ley farming system of South Australia, but by no means do they account for all permutations of factors.

5.2 Bioassay Methodology

Graded seed was obtained by sieving seed of *M. truncatula* cv. Paraggio through a 1.7 mm square-mesh sieve and then through a 1.4 mm square-mesh sieve. Seed retained on the top sieve and the seed which passed through the smaller mesh sieve was discarded. A rectangular sheet of filter paper 120 x 200 mm was marked with a line 80 mm from one of the long edges. Forty seeds were placed on the filter paper along the pencil line, approximately equidistant from each other, then another sheet of filter paper was placed over the original sheet. The sheets of paper were carefully wound around a 60 mm diameter bottle and retained in position with two rubber bands so that the seeds were all aligned in a horizontal plane around the circumference of the plastic bottle, 80 mm from the base.

Most of the work reported here was undertaken with the medic cultivar Paraggio because this medic is currently recommended for sowing over extensive areas of south eastern Australia, including South Australia (Lake and Crawford 1985). Initially selected for aphid tolerance, Paraggio produces greater quantities of herbage than the superseded cultivars including Hannaford and Jemalong and also it has superior levels of seed production (Amor *et al.* 1985, 1986).

Bioassay assemblies are illustrated in Plate 5.1. Each assembly was placed in a wide-mouthed, 1000 ml capacity, glass jar (Agee utility) and irrigated with leachate which was slowly poured over the longitudinal axis of the plastic bottle to ensure even distribution through the paper. After about two minutes, excess leachate which had drained from the paper was removed from the jar and re-applied. Any solution which drained from the paper this second time was discarded. This procedure ensured that the filter papers were evenly moistened with solution. A control bioassay was prepared using deionized water instead of straw leachate.

The completed bioassay assemblies were stored in the dark inside a closed plastic box which also contained a shallow pool of water so as to maintain humidity and hence minimized drying of the filter papers. After the bioassays were held at 20°C for three days, ten seedlings were removed from each assembly and the lengths of the radicle and hypocotyl of each seedling were measured, then these seedlings were discarded. Similar measurements were conducted over the next two days. The hypocotyl was defined as the seedling axis from the junction of the cotyledons to the point along the longitudinal axis where radial contraction first occurred.

The straw used in these studies had been collected from farms shortly after grain harvest following the 1983 growing season. An exception was Galleon barley which was grown at the Waite Agricultural Research Institute, Urrbrae, using commercial methods. Grain was reaped from the crop of Galleon on November 28, 1983 and straw was collected on December 2, 1983, thus there was little weathering of the stubble. All straw samples were stored in a room at ambient temperatures for a period of approximately 14 months before use in bioassays. Straw samples were ground in a Wiley mill fitted with a 7 mm square-mesh concave then 8 g portions of chaff were soaked in 200 ml of deionized

water (4:100 W/V) for 16 hours, at a temperature of 2°C. At this temperature microbial decomposition of the straw was minimized. The resultant leachate, after decanting, was filtered through a Whatman No.1 grade qualitative filter paper and then sterilized by passing it through a Millipore filter with 0.45 μm pore size. Fifty ml of solution was used to irrigate each bioassay assembly. In some experiments, solutions were bulked together to ensure sufficient volume.

Comparison of treatment effects was made using a one-way analysis of variance, unless otherwise indicated.

5.3 Experimental

5.3.1 Inhibition of medic seed germination by leachates from cereal straw

The effects of leachates on the germination percentage of seeds has commonly been used as an assessment of phytotoxicity of leachates (Rice 1974, 1979). In these previous studies, germination was conducted in petri dishes after moistening the medium, usually filter paper, with leachate (Guenzi *et al.* 1967; Leather and Einhellig 1985). In some cases the leachates failed to affect germination but retarded seedling elongation, thus effects on germination are not a complete guide to phytotoxicity. This bioassay evaluation of possible phytotoxic effects of leachates on annual medic germination may contribute to a greater understanding of observations in Field Surveys 1, 2 and 3 where medic seedling densities were negatively related to high concentrations of straw.

Materials and Methods

Leachates were obtained from straw of ten cereal cultivars which had been collected from different localities shortly after grain harvest. Two samples of Coorong triticale came from widely separated regions.

Cereal cultivars used in the evaluation

Gabo (*Triticum aestivum*)	Weeah (*Hordeum vulgare*)	S.A. rye (*Secale cereale*)
Halberd "	Schooner "	Avon (*Avena sativa*)
Oxley "	Galleon "	Currency (*X Triticosecale*)
		Coorong"A" "
		Coorong"B" "

Petri dishes which contained a single Whatman No.1, qualitative filter paper and 100 seeds of Paraggio barrel medic were moistened with 5 ml of leachate, but control dishes were moistened with deionized water. There were eight replicates. After storage in the dark at 20°C for four days, the number of germinated seeds in each dish were counted. Germination was defined as protrusion of the radicle to a length exceeding the longest dimension of the seed. There was insufficient range in the germination data to warrant transformation before analysis of variance, according to the rationale of Snedecor and Cochran (1967).

In a second experiment, leachate was prepared from Galleon barley straw and 5 ml aliquots of this leachate, or deionized water, were added to petri dishes containing two No.1, qualitative filter papers and 100 medic seeds. Nine cultivars of *Medicago* spp. (see Table 5.1) were compared using two replicates for each treatment. A factorial analysis of variance was used to compare treatment means.

Results

There were no differences in the germination percentages of *M. truncatula* cv. Paraggio caused by any of the 11 leachates when compared with the water control. The mean germination percentage was 90.4 per cent and the amount of hard seed was 6.2 per cent, thus adjusting for hard seed content, the corrected germination percentage was 96.4 per cent. The F value computed in the analysis of variance was only 0.436 and the data have not been presented here.

Data which describe the effects of leachate from Galleon barley chaff on germination of nine cultivars of annual medic are shown in Table 5.1. Analysis of variance showed that the main effect of leachate on seed germination was highly significant ($P<0.01$). Leachate reduced mean seed germination by slightly less than 10 per cent. The main effect of medic cultivar was significant ($P<0.05$). Although the interaction between leachate and medic cultivar was not significant in this analysis, the germination of Paraggio was least affected by leachate and germination of seed of Harbinger was reduced the

most (over 30 per cent). The low germination percentage of Circle valley (Table 5.1) was the result of low seed viability rather than a high proportion of hard seed.

TABLE 5.1: Germination percentages of eight medic cultivars, 4 days after moistening with water or with a leachate from Galleon barley straw.

Medic cultivar	Water	Leachate	% of Maximum †
Serena (*M. polymorpha*)	97.1	91.7	94.4
Circle Valley (*M. polymorpha*)	82.1	72.0	87.7
Sava (*M. scutellata*)	98.3	89.2	90.8
Paraggio (*M. truncatula*)	97.9	97.4	99.5
Jemalong (*M. truncatula*)	98.9	87.9	88.9
Sephi (*M. truncatula*)	95.7	90.9	95.0
Cyprus (*M. truncatula*)	97.9	87.7	89.6
Harbinger (*M. littoralis*)	100.0	67.4	67.4
Paraponto (*M. rugosa*)	98.0	95.5	97.5
Mean	96.2	86.6	
Leachate ** LSD (P=0.05) = 2.9			
Medic cultivar * LSD (P=0.05) = 10.5			
Interaction not significant			

† Germination with leachate as a proportion of germination with water

5.3.2 Effects of leachates from cereal species and cultivars on medics

Published results of experiments indicate that different cereal cultivars, when tested in bioassays, may give a range of reactions from a single test genotype. Guenzi *et al.* (1967) used bioassays to examine leachates from stubble of nine cultivars of wheat. These leachates exhibited differing capacities to retard growth of wheat seedlings. Kimber (1967) found that leachate from straw of three varieties of wheat caused different levels of inhibition of root growth of both Early Burt oats and Gabo wheat seedlings. Lovett and Jessop (1982) demonstrated that leachate from barley residue was more effective than leachate from wheat or oats in reducing the root growth of three day old wheat seedlings. In addition to the effects of cereal genotype, the conditions under which the crop is grown may influence the phytotoxicity of materials present in the residue (Harris and Kimber 1983; Putnam 1985). In particular, the environmental stresses that crops encounter, included nitrogen deficiency, high salt levels and drought, may increase subsequent development of phytotoxicity (del Moral 1972).

The aim of this group of experiments was to examine possible variation in inhibition of medic growth caused by leachates from straws of different cereal species and subsequently to assess the reactions of seedlings to leachate from commonly-grown cultivars of the cereal species considered to be most phytotoxic. Leachates from cereal crops grown under two regimes of nitrogen fertilizer were also compared.

Materials and Methods

Leachates were prepared from straw of four cereal species and the solutions were tested using the bioassay methods described above (section 5.2). As these techniques were in a developmental stage, only total seedling length of Paraggio medic was measured over three days, commencing three days after wetting.

The results from this preliminary experiment prompted a second, more detailed, investigation of the phytotoxicity associated with leachates from residues of three barley cultivars viz. Weeah, Galleon and Schooner, and both hypocotyl and radicle lengths were measured in the bioassay.

A third bioassay was conducted with leachates obtained from six commercial cultivars of barley (Table 5.4). The straw for this experiment originated from field plots which had received either 10 kg/ha or 70 kg/ha nitrogen (designated L or H treatment) applied as ammonium nitrate before sowing. All straw was collected from a site at Arthurton, Yorke Peninsula, (South Australia) in late December 1985, on the same day that grain was reaped. The pH of the fresh leachate from each treatment was measured using a Jenco pH meter, as extremes of acidity were suspected as the causes of impaired seedling development. The results from the 12 Straw x Nitrogen bioassays were compared with those from a distilled water control using a one-way analysis of variance.

Results

In both bioassays the total seedling length of Paraggio was reduced by leachates derived from Galleon and Weeah barleys, especially by leachate from Weeah (Tables 5.2 and 5.3). Aqueous extracts from South Australian rye, Gabo wheat and Coorong triticale, in contrast, did not consistently affect seedling elongation. Hypocotyl extension was unaffected but radicles were sensitive to aqueous leachates.

On Day 3 of the third bioassay, the mean radicle lengths of seedlings in all treatments which received leachate were shorter than the control (Table 5.4). Hypocotyl length was unaffected by the presence of leachate throughout the experiment except for one treatment on Day 3 but this did not persist. As a consequence, total seedling lengths were all less than the control on Day 3, but not statistically significant in two treatments. Over the next two days the inhibition of radicle elongation appeared to abate so that by Day 5 only two treatments were significantly different from the control.

TABLE 5.2: Effects of leachates from a range of cereal species and cultivars on total seedling length of Paraggio barrel medic.
(Measured on three successive days).

Leachate source	Day 3	Day 4	Day 5
		(mm)	
Control	28.4	57.9	80.2
Rye (*Secale cereale*)	27.7	59.3	89.3
Weeah (*Hordeum vulgare*)	14.2	29.4	43.7
Galleon (*Hordeum vulgare*)	23.9	47.1	68.4
Gabo (*Triticum aestivum*)	29.2	54.9	79.3
Coorong (*X Triticosecale*)	25.2	50.4	77.3
Signif. of Diff.	**	**	**
LSD (P= 0.05)	4.2	5.6	6.1

** P<0.01

Any differences in medic seedling length attributable to previous nitrogen nutrition regimes of the barley were transitory and inconsistent between barley cultivars.

The pH values measured in the leachates ranged between 6.9 and 7.5 with a mean of 7.1 and the pH of these solutions was unlikely to have any influence on seed germination and seedling development. Le Tourneau *et al.* (1956) claimed their experiments showed that pH of aqueous solutions over the range 4.9 to 6.8 were not responsible for inhibition of germination and root growth of pea and wheat. Furthermore, Kimber (1967) determined that the pH of aqueous solutions from a range of straw types varied between 4.7 and 5.9 but that there was no obvious relationship between different levels of toxicity and pH. This conclusion was supported by the results of Patterson (1981) and Blum *et al.* (1985a).

TABLE 5.3: The effects of leachates from three barley cultivars on elongation of seedlings of Paraggio barrel medic.

Barley cultivar	Day 3	Day 4			Day 5		
	Total	Hypocotyl	Radicle	Total	Hypocotyl	Radicle	Total
			(mm)				
Control	58.7	33.9	52.9	86.8	41.5	58.9	100.4
Weeah	29.1	30.6	17.3	47.9	40.4	25.5	65.9
Galleon	48.4	32.5	42.9	75.4	42.2	51.2	93.4
Schooner	46.2	31.0	44.2	75.2	41.6	58.3	99.9
Signif. of Diff.	**	ns	**	**	ns	**	**
LSD (P=0.05)	5.4		4.0	6.0		4.3	6.5

*P< 0.05; ** P<0.01; ns, not significant

TABLE 5.4: Effects of leachates from six barley cultivars, grown under two nitrogen regimes, on the elongation of seedlings of *M. truncatula* cv. Paraggio. (H= high nitrogen, L=low nitrogen).

(Bold figures are significantly different to the relevant control treatment below)

Barley cultivar	Day 3				Day 4				Day 5			
	Hypocotyl		Radicle		Total		Hypocotyl		Radicle		Total	
	H	L	H	L	H	L	H	L	H	L	H	L
	(all data in mm)											
O Connor	11.8	12.7	**27.6**	**29.5**	**39.4**	**42.2**	22.1	25.9	**41.3**	45.8	**63.4**	71.7
Forrest	11.8	12.3	**26.4**	**28.0**	**38.2**	**40.3**	24.8	25.9	44.6	48.1	69.4	74.0
Stirling	12.0	11.7	**28.7**	**25.6**	**40.7**	**37.3**	21.7	25.6	**43.7**	**43.7**	**65.4**	69.3
Galleon	**10.9**	11.7	**29.4**	**28.9**	**40.3**	**40.6**	25.9	26.6	46.2	45.3	72.1	71.9
Weeah	14.7	14.3	**27.3**	**28.6**	**42.0**	42.9	24.7	26.6	**39.9**	**38.1**	**64.6**	**64.7**
Clipper	12.2	12.8	31.0	**28.9**	43.2	**41.7**	24.1	24.3	48.9	**41.7**	73.0	**66.0**
Control	13.5		33.5		47.0		25.6		48.4		74.0	
Signif. of Diff.	**		**		**				**		**	
LSD (P=0.05)	1.8		3.2		4.3		ns		3.9		5.8	

Barley cultivar	Day 5			
	Hypocotyl		Radicle	
	H	L	H	L
O Connor	34.2	34.4	57.6	56.2
Forrest	32.6	35.6	**50.1**	60.0
Stirling	34.4	35.5	56.6	**50.3**
Galleon	34.7	34.1	57.7	56.7
Weeah	35.7	37.7	56.8	58.3
Clipper	32.6	36.1	61.0	58.0
Control	37.3		59.1	
Signif. of Diff.			*	
LSD (P=0.05)	ns		6.5	

Barley cultivar	Day 5		
	Total		
	H	L	
O Connor	91.8	90.6	
Forrest	**82.7**	95.6	
Stirling	91.0	**85.8**	
Galleon	92.4	90.8	
Weeah	92.5	96.0	
Clipper	93.6	94.1	
Control	96.4		
Signif. of Diff.	**		
LSD (P=0.05)	6.5		

5.3.3 Medic cultivar responses to leachates from straw of Weeah and Galleon barley

Results from section 5.3.2 indicated that leachate from straw of Weeah barley was highly phytotoxic: therefore residues of Weeah straw were utilized for experiments in which a larger number of medic genotypes were investigated. Although Galleon barley residues had less phytotoxicicity than those from Weeah, it was also included here as it is the most widely grown variety of barley in South Australia. These bioassays compared the effects of leachates on a range of medic cultivars which were representative of all commonly-grown annual medic species in South Australia. Further agronomic descriptions of these medic cultivars are given by Lake and Crawford (1985).

Materials and Methods

Six medic cultivars (Circle Valley, Jemalong, Paraggio, Sapo, Sava, Serena), which represented four commonly-grown species, were used in a bioassay with leachate obtained from Weeah barley straw. Paraggio and Jemalong barrel medics were mechanically graded for seed size and the other cultivars were hand selected to ensure uniformity of size.

In a second experiment, leachate from Galleon barley straw was used to assess the reactions of another selection of medic cultivars including representatives from each *Medicago* species used in the first bioassay plus *M. littoralis* . Measurement of seedling growth was unavoidably precluded before Day 4. These two straw samples of Weeah and Galleon were the same as these used in the first two germination tests in section 5.3.2. For each medic cultivar, results from the leachate treatment were compared with the control using Student's *t*-Test.

Results

Leachate from Weeah barley

The data in Table 5.5 demonstrate the disparate effects of leachate on the elongation of young seedlings of different medic cultivars. Seedling development of Sava and Sapo was comparatively slow, therefore it was often not possible to distinguish the junction between the hypocotyl and radicle so only total lengths were recorded. The cultivar Sapo failed to show any deleterious effects from leachate of Weeah barley. The *M. truncatula* cultivars Paraggio and Jemalong were consistently retarded in radicle extension throughout. Extension of young roots of Circle Valley was retarded by leachate on Day 5 but not before then.

Leachate from Galleon barley

Results from this bioassay are shown in Table 5.6. The germination progress of Sava, Paraponto and Harbinger medics was markedly retarded by the presence of leachate, consequently measurements could not be made early in the experiment and no observations at all could be made on seedlings of Paraponto because of gross retardation of germination. The extent of inhibition of development of seedlings of Sava and Harbinger medics is indicated by the gross difference in results of leachate and control treatments on Day 6. The other five medic cultivars exhibited retardation of seedling elongation, especially impaired radicle extension. A transient stimulation of hypocotyl growth in the presence of leachate occurred in the cultivar Serena on Day 5 but this response is atypical. An error occurred in counting the original number of seeds of Sephi medic so there were insufficient seedlings for measurement of seedling development on Day 6.

The data from Tables 5.5 and 5.6 suggests that the leachate derived from Weeah barley was more phytotoxic than the leachate from Galleon barley particularly when radicle lengths are compared 4 or 5 days after moistening seeds. This supports previous results from section 5.3.2.

The trends of radicle growth during the course of the two bioassays are shown in Figure 5.1. Differences between species were more evident than differences between cultivars of the same species.

TABLE 5.5: The effects of leachate from Weeah barley straw on seedling elongation of a range of annual medics.

Cultivar and species		Day 3			Day 4			Day 5		
		Hypocotyl	Radicle	Total	Hypocotyl	Radicle	Total	Hypocotyl	Radicle	Total
						(mm)				
Serena	L	14.8	17.4	32.2	28.3	26.0	54.3	30.8	29.4	60.2
M. polymorpha	C	15.9	23.4	39.3	27.7	38.8	66.5	37.7	55.5	93.2
Signif. of Diff.		ns	ns	ns	ns	**	**	*	**	**
Circle Valley	L	16.7	20.5	37.2	36.5	29.6	64.1	38.3	26.5	66.8
M. polymorpha	C	20.4	24.2	44.6	33.0	31.6	64.9	40.0	42.9	82.9
Signif. of Diff.		ns	ns	*	ns	ns	ns	ns	**	*
Sava	L	-†	-†	9.0	-†	-†	29.2	15.4	36.8	52.2
M. scutellata	C	-†	-†	15.3	16.7	31.7	48.4	20.3	54.1	74.4
Signif. of Diff.				ns			**	ns	**	**
Paraggio	L	13.7	12.5	26.2	24.4	20.0	44.4	30.3	21.1	51.4
M. truncatula	C	21.0	31.7	52.7	29.2	47.1	76.3	36.3	58.8	95.1
Signif. of Diff.		**	**	**	**	**	**	**	**	**
Jemalong	L	17.0	14.6	31.6	32.7	22.2	54.9	39.8	25.9	65.7
M. truncatula	C	21.1	30.7	51.8	31.0	43.0	74.0	39.5	52.2	91.7
Signif. of Diff.		**	**	**	ns	**	**	ns	**	**
Sapo	L	-	-	21.5	-	-	31.8	22.2	34.2	56.4
M. rugosa	C	-	-	20.6	-	-	40.6	21.1	31.7	52.8
Signif. of Diff.				ns			ns	ns	ns	ns

L = Leachate treatment , C = Control: * P< 0.05; ** P< 0.01; ns, not significant.

† No clear demarcation between hypocotyl and radicle.

TABLE 5.6: The effects of leachate from Galleon barley straw on seedling elongation of a range of annual medics.

Cultivar and species		Day 4			Day 5			Day 6		
		Hypocotyl	Radicle	Total	Hypocotyl	Radicle	Total	Hypocotyl	Radicle	Total
						(mm)				
Serena	L	35.7	25.4	61.1	52.4	34.7	87.1	54.5	26.6	81.1
M. polymorpha	C	32.4	46.4	78.8	42.9	51.7	94.6	48.7	49.8	98.5
Signif. of Diff.		ns	**	**	*	**	ns	ns	**	ns
Circle Valley	L	39.4	20.3	59.7	49.2	29.6	78.8	44.0	20.0	64.0
M. polymorpha	C	41.7	39.2	80.9	52.6	36.7	89.3	59.8	48.7	108.5
Signif. of Diff.		ns	**	**	ns	ns	ns	ns	**	**
Sava	L	-	-	-	-	-	-	-†	-†	37.2
M. scutellata	C	19.8	42.1	61.9	37.0	64.0	101.0	46.4	67.3	113.7
Signif. of Diff.										**
Paraggio	L	31.4	35.7	67.1	46.2	44.2	90.4	53.3	50.7	104.0
M. truncatula	C	40.0	56.8	96.8	47.0	55.4	102.4	55.4	70.5	125.9
Signif. of Diff.		**	**	**	ns	**	*	ns	**	**
Jemalong	L	29.0	31.3	60.3	39.1	36.8	75.9	47.9	45.3	94.6
M. truncatula	C	40.5	54.6	94.8	48.9	62.6	111.5	52.7	62.1	112.8
Signif. of Diff.		**	**	**	**	**	**	ns	**	**
Sephi	L	27.9	32.2	60.1	42.7	36.9	79.6	-	-	-
M.truncatula	C	41.1	56.5	97.6	52.1	60.2	112.3	52.0	69.3	121.3
Signif. of Diff.		**	**	**	ns	**	**			
Harbinger	L	-	-	-	-	-	-	23.6	20.0	43.9
M.littoralis	C	30.9	39.1	70.0	40.6	38.9	79.5	43.8	42.4	86.2
Signif. of Diff.								**	**	**
Paraponto	L	-	-	-	-	-	-	-	-	-
M. rugosa	C	29.2	48.4	77.6	39.8	51.7	91.5	-	-	-

L = Leachate treatment ; C = Control; * P< 0.05; ** P< 0.01; ns, not significant.

† No clear demarcation between hypocotyl and radicle.

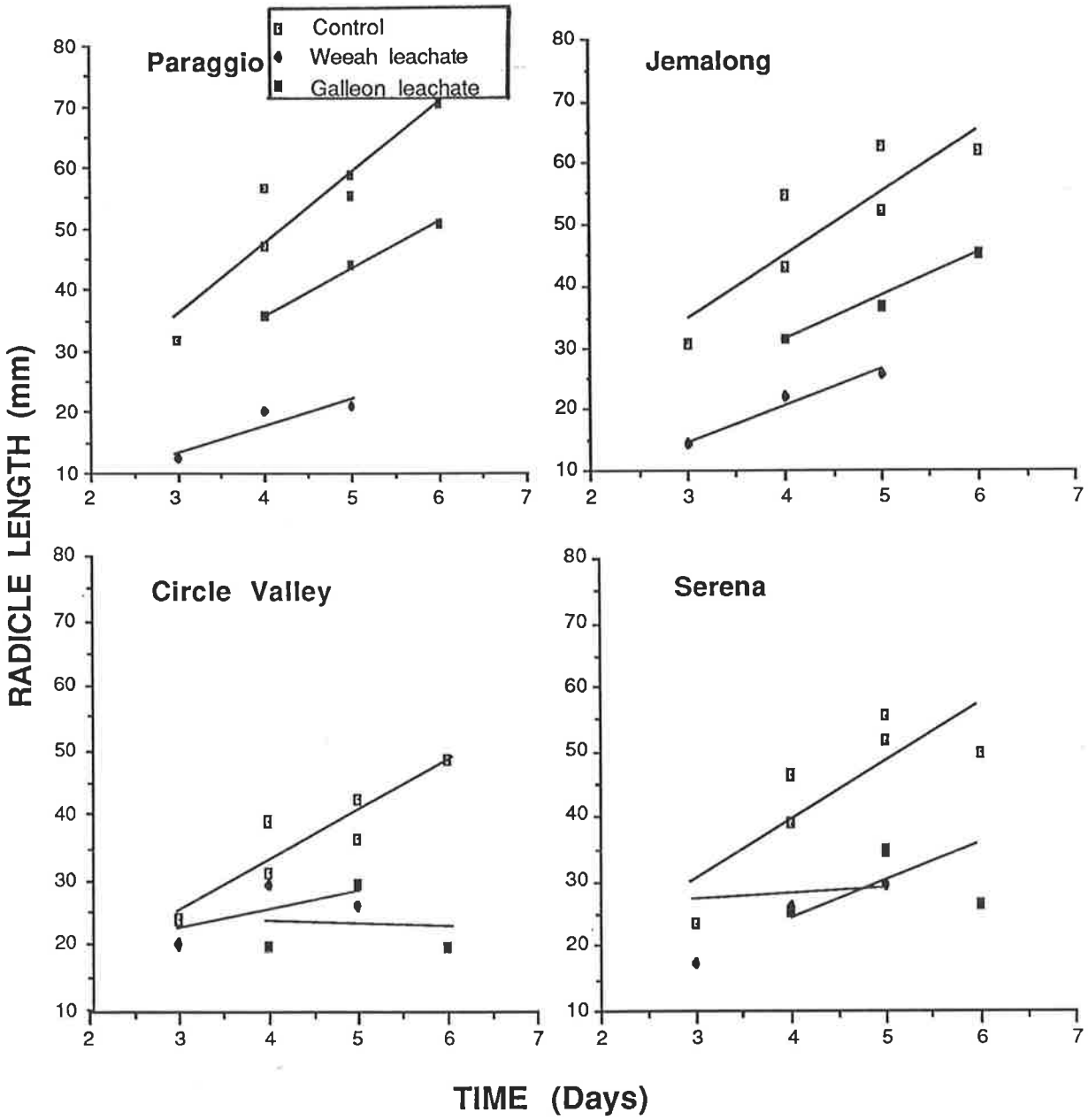


FIGURE 5.1: Trends in radicle growth of medic seedlings in bioassays with leachates from Weeah and Galleon barleys, shown for two *M. truncatula* cultivars (Paraggio and Jemalong) and two *M. polymorpha* cultivars (Circle Valley and Serena). Lines fitted by linear regression analysis. For each graph, the data from controls in both bioassays have been combined so a single trend is shown.

5.3.4 Assessment of leachates progressively obtained from fresh straw

A study of the research literature indicates that little attention has been given to the methods used to leach straw. Most commonly the straw was immersed in water for several hours or more, for example Shilling *et al.* (1985). Liberation of phytotoxic compounds from straw may well follow different patterns depending on the rate that water penetrates the inter- and extra-cellular spaces of the straw and the rate at which compounds from straw enter into aqueous solution. Consequently the objective of the experiment described below was to obtain progressive leachates from a single straw sample using less-vigorous leaching methods which simulated repeated light rainfall. The aqueous solutions obtained with this technique were subsequently compared in a bioassay.

Materials and Methods

A watertight base was constructed, using a sheet of polystyrene covered with a layer of polypropylene, to fit inside the lower section of a 250 mm diameter, 1.25 mm square-mesh sieve. A single 7 mm diameter hole was drilled through both layers of plastic and a length of rubber tubing attached to this drain. The sieve was loosely packed with 19.6 g of Galleon barley chaff which had been milled in a Wiley mill as described previously (see section 5.2). This gave a straw concentration equivalent to 400 g/m^2 within the sieve, a concentration close to values commonly observed in the field.

Each hour the straw was sprayed with a hand-operated atomizer filled with rainwater. Sufficient water was applied each time so that 100 ml of solution was drained from the base, a volume equivalent to 2 mm of rainfall over the area of the sieve. The straw and sieve were weighed at the commencement of the experiment and after every irrigation to measure the amount of water retained by the straw. Sequential leachates obtained after irrigation were stored on ice until all leachates had been collected. Ten leachates (numbered 1 to 10) were collected then the straw was left for 16 hours, after which a further two leachates were obtained with one hour between collections (leachates numbered 11 and 12). The twelve leachates were filtered, sterilized and tested using the bioassay techniques as described in section 5.2.

Results

On Day 3 of the bioassay the first five leachates caused a similar level of inhibition of radicle elongation, so their lengths were considerably shorter than that of the control (Figure 5.2). While there was a significant difference between the effects of the first five treatments and the next five, Figure 5.2 suggests that there was a linear response in radicle length to leachate number. In consequence, these effects progressively diminished with leachates collected later in the day. Leachates collected at hours 27 and 28 exhibited enhanced phytotoxicity commensurate with effects from leachates collected in the first few hours. On Days 4 and 5 in the bioassay, the first leachate collected was less phytotoxic than the leachate obtained one hour later. Subsequent leachates again exhibited a trend of reduced phytotoxicity related to the sequence of collection. The magnitude of the deleterious effects of leachates should be noted, for on Day 5 radicle growth with the first two leachates and those obtained on the second day were approximately half the length of the control. These results suggest that there was limited opportunity for liberation of phytotoxins during the initial leaching and that the quantities of phytotoxins diminished during the latter leachings.

The development of hypocotyls of seedlings was generally unaffected by leachates, except for the first leachate obtained on the second day which caused a significant reduction in hypocotyl extension on Day 3 and Day 5 (Table 5.7).

The straw absorbed 71 g of water during the initial wetting and retained a further 37 g of water when the straw was leached a second time. Over the next ten irrigations the straw continued to absorb approximately equal amounts of water, 5.6 g on each occasion. Finally when all irrigations had been completed the straw had absorbed water (144.4 g) equal to seven and a half times the original weight of air-dry straw, or equivalent to about 3 mm of rainfall.

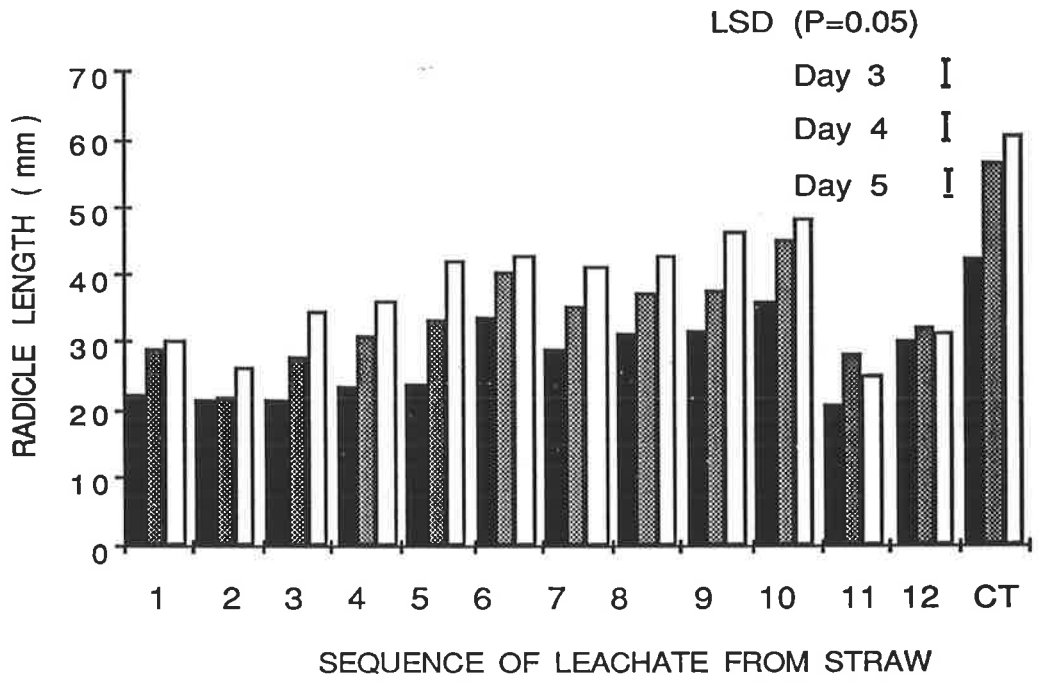


FIGURE 5.2: Effects of a sequence of leachates from straw of Galleon barley on the elongation of radicles of barrel medic cv. Paraggio. CT is the control treatment.

There was a 16 hour pause between collection of samples 10 *and 11, otherwise sequential samples were separated by an interval of one hour.*

Duration of bioassay
Day 3
Day 4
Day 5

5.3.5 Effects of straw weathering in the field on phytotoxicity of leachates

Several studies have shown that weathering of straw reduces its phytotoxicity (Horricks 1969; Guenzi *et al.* 1967; Wang *et al.* 1967; Kimber 1967, 1973a; Cochran *et al.* 1977, 1982). Guenzi *et al.* (1967) found the greatest phytotoxicity of aqueous leachates from wheat and oat straw (determined in bioassays) occurred at or near grain harvest but phytotoxicity had dissipated after four weeks of straw decomposition in the field. More recently Harris and Kimber (1983) concluded that in general the peak of phytotoxicity occurs in the first day or two of decomposition and may persist for weeks. The level of inhibitor may also depend on the stage of maturity of plant material when samples are extracted. Green vegetation is more potent than dry, mature plant residues (Le Tourneau *et al.* 1956; Kimber 1967).

Materials and Methods

Schooner barley straw from Field Experiment 1 (Effects of straw concentration on natural regeneration and productivity of an annual medic pasture) was collected each 28 days (originally 4 t/ha of straw) and utilized in this bioassay, except for samples from the ninth harvest which were inadvertently discarded. Dried green herbage (oven dried) from self-sown barley collected on Harvest 10 (Oct. 23) was also included. All the residues were ground in a Wiley mill before leachates were prepared.

Results

Weathering of barley residues in the field, reduced the phytotoxicity of leachates. Schooner straw collected on Feb.14 and Mar.13 contained soluble phytotoxic compounds, as indicated by retardation of radicle elongation in this bioassay but straw weathered beyond Mar.13 did not have this capacity (Figure 5.3). The leachate originating from green, self sown barley was also phytotoxic to young medic roots.

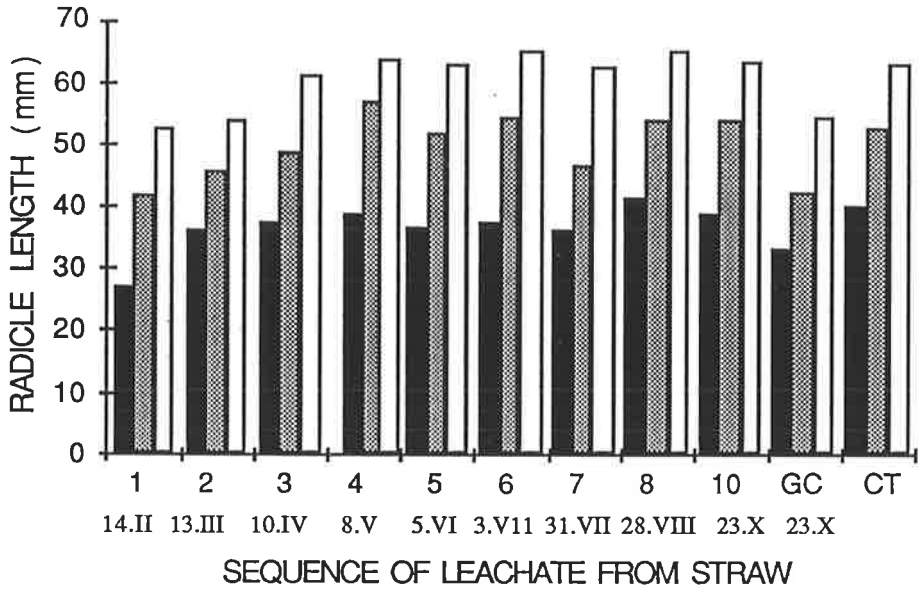


FIGURE 5.3: Retardation of radicle extension caused by leachates originating from partly-weathered straw or from green cereal herbage (GC). CT is the control treatment.

Straw was sampled at 28 day intervals, sample dates indicated.

Duration of bioassay

Day 3

Day 4

Day 5

TABLE 5.7: The effects of leachates from Galleon barley straw (irrigated at hourly intervals) on seedling elongation of *M. truncatula* cv. Paraggio.

Leachate in sequence	Day 3			Day 4			Day 5		
	Hypocotyl	Radicle	Total	Hypocotyl	Radicle	Total	Hypocotyl	Radicle	Total
					(mm)				
Control	22.0	42.4	64.4	32.5	56.3	88.8	38.0	60.8	98.8
1	20.4	22.4	42.8	33.9	29.0	62.9	42.6	30.1	72.7
2	23.5	21.5	45.0	33.5	21.8	55.3	40.1	26.1	66.2
3	18.8	21.6	40.4	34.1	28.0	62.1	39.5	34.2	73.7
4	19.8	23.6	43.4	32.2	31.0	63.2	41.2	36.1	77.3
5	19.5	23.8	43.3	34.4	33.3	67.7	40.0	42.1	82.1
6	21.0	33.8	54.8	33.4	40.5	73.9	38.8	43.0	81.8
7	21.1	29.1	50.2	33.9	35.1	69.0	41.1	41.1	82.2
8	21.4	31.2	52.6	30.7	37.6	68.3	38.6	42.8	81.4
9	19.7	31.8	51.5	30.9	38.0	68.9	39.0	46.6	85.6
10	22.9	36.2	59.1	34.0	45.3	79.3	40.6	48.4	89.0
					16 hours interval				
11	18.1	20.8	38.9	32.8	28.4	61.2	31.6	25.5	57.1
12	20.1	30.0	50.1	35.2	32.0	67.2	41.4	31.2	72.6
Signif. of Diff.	**	**		ns	**		**	**	
LSD (P=0.05)	2.6	3.3			3.5		3.4	3.5	

** P< 0.01; ns, not significant

5.3.6 Microbiological filtration of leachates and its effect on phytotoxicity

Phytotoxins liberated from plant residues may be degraded by microbial activity so these compounds are metabolized to non-toxic or less toxic compounds. Greenwood and Kimber (1967) using a bioassay system with wheat seedlings found that the addition of a dilute solution of soil microflora to moist straw of *Bromus mollis*, subsequently attenuated the effects of leachate from the straw. Bacteria can play an important role in the production of allelochemicals from plant material (Lovett 1987) but much more needs to be learnt about this aspect.

In the study described below, the objective was to assess the impact that microbial contamination may have on bioassay results. This aim was combined with an examination of the effects of leachates from straw of different particle sizes.

Materials and Methods

Chaff of Galleon barley which had been ground in a Wiley mill was sequentially sieved using 1.4 mm and 0.5 mm square-mesh sieves. Chaff which had passed through the 0.5 mm sieve and that retained on the 1.4 mm sieve as well as the original mix were used to prepare three leachates. The method of leachate preparation was modified by adding 1 ml of a water slurry of soil to each chaff incubation and the solution was held at 20°C instead of 2°C for 16 hours, after which the solution was filtered but not sterilized (non-sterile treatment). This change in technique was designed to promote microbial degradation of compounds leached from the straw. Conventional leachates were also prepared using the methods described in the methodology section 5.1 (sterile treatment). The seed was surface sterilized by shaking with 95% ethanol for 15 seconds, then with 0.2% $HgCl_2$ for 3 minutes, followed by several rinses with sterile water, also the jars for these treatments were surface-sterilized by swabbing with alcohol. The osmotic potentials of the three conventionally prepared (sterile) solutions were measured using a Wescor thermocouple psychrometer.

Treatments were replicated three times and a factorial two-way analysis of variance was used for statistical examination of the bioassay results.

Results

Data from this experiment (Table 5.8) confirm the observation that cereal crop residues may contain deleterious, soluble compounds. Plate 5.1 shows representative seedlings from the control and sterile leachate treatments. The particle size of straw used during the leaching process caused little difference in phytotoxicity. Leachate derived from coarse particles was the most phytotoxic treatment early in the bioassay but by the fifth day this leachate had equivalent effects to the other two leachate treatments. Early retardation of hypocotyl elongation, caused by the leachates, did not persist beyond Day 3.

The sterile leachate was responsible for significantly greater retardation of radicle extension compared with non-sterile leachate (Table 5.8). This consistent disparity between these treatments indicates that phytotoxic compounds, initially present in solutes, were rapidly decomposed to less-active compounds by microbial activity. The presence of a significant interaction in the analysis was largely due to the absence of any effect of sterilization in the controls. This should also be taken into consideration when comparing the mean effects of sterilization because if the control data are removed from the calculation then the main effects of sterilization become larger than those shown in Table 5.8.

The osmotic potentials of the solutions were 1.2, 1.1 and 2.1 bars for the coarse, fine and mixed particle treatments respectively. The magnitude of these values is too low to be responsible for large differences in responses by test plants. Putnam and Duke (1978) have noted that the osmotic pressure of aqueous solutions are often not considered in interpretation of bioassay results but caution has been advised by Anderson and Louchs (1966) who believed that the osmotic factor could have a major effect on bioassay results. The conclusions of Guenzi and McCalla (1962) and Greenwood and Kimber (1967) do not support the contention that osmotic pressure may be important. Bell (1974) found that solutions with osmotic concentrations exceeding 1.7 atm were able to suppress growth of radicles of *Bromus rigidus* and he believed that bioassay results obtained from leachates with higher osmotic potential than this should be interpreted with care. However, the species of seed used in the bioassay may affect the response to osmotic pressure.

TABLE 5.8: Effects of straw particle size during leaching, and sterilization of leachate on elongation of seedlings of Paraggio medic.

(Figures in parentheses are the means of the non-sterile and sterile treatment pair)

Straw particle size		Day 3			Day 4			Day 5		
		Hypocotyl	Radicle	Total	Hypocotyl	Radicle	Total	Hypocotyl	Radicle	Total
					(all data in mm)					
Control	NS†	22.0	36.2	58.2	31.7	48.3	80.0	42.5	56.4	99.0
	S††	19.6	37.5	57.1	29.5	51.0	80.5	38.6	58.2	96.8
		(20.8)	(36.9)	(57.7)		(49.7)	(80.3)		(57.3)	(97.9)
Coarse	NS†	18.6	18.2	36.8	32.2	24.5	56.6	43.8	30.8	56.6
	S††	18.1	15.5	33.6	29.3	18.1	47.3	38.5	22.0	60.4
		(18.4)	(16.9)	(35.2)		(21.3)	(52.0)		(26.4)	(67.5)
Fine	NS†	20.0	27.4	47.4	34.7	37.9	72.6	45.1	42.2	87.3
	S††	16.6	11.1	27.7	30.2	13.7	43.9	38.0	16.6	54.6
		(18.3)	(19.3)	(37.6)		(25.8)	(58.3)		(29.4)	(71.0)
Mixed	NS†	18.8	26.7	45.5	34.3	33.6	67.9	38.3	32.7	71.0
	S††	16.8	13.9	30.8	29.7	15.8	45.5	38.1	19.3	57.4
		(17.8)	(20.3)	(38.2)		(24.7)	(56.7)		(26.0)	(64.2)
Signif. of Diff.		*	**	**	ns	**	**	ns	**	**
LSD (P = 0.05)		2.1	1.8	1.5		1.5	4.9		8.4	11.1
Non-sterile		19.9	27.1	47.0	33.2	36.1	69.3	42.4	40.5	83.0
Sterile		17.8	19.5	37.3	29.7	24.7	54.3	38.3	29.0	67.3
Signif. of Diff.		**	*	*	*	**	**	*	*	*
Interaction		ns	**	**	ns	**	**	*	*	*

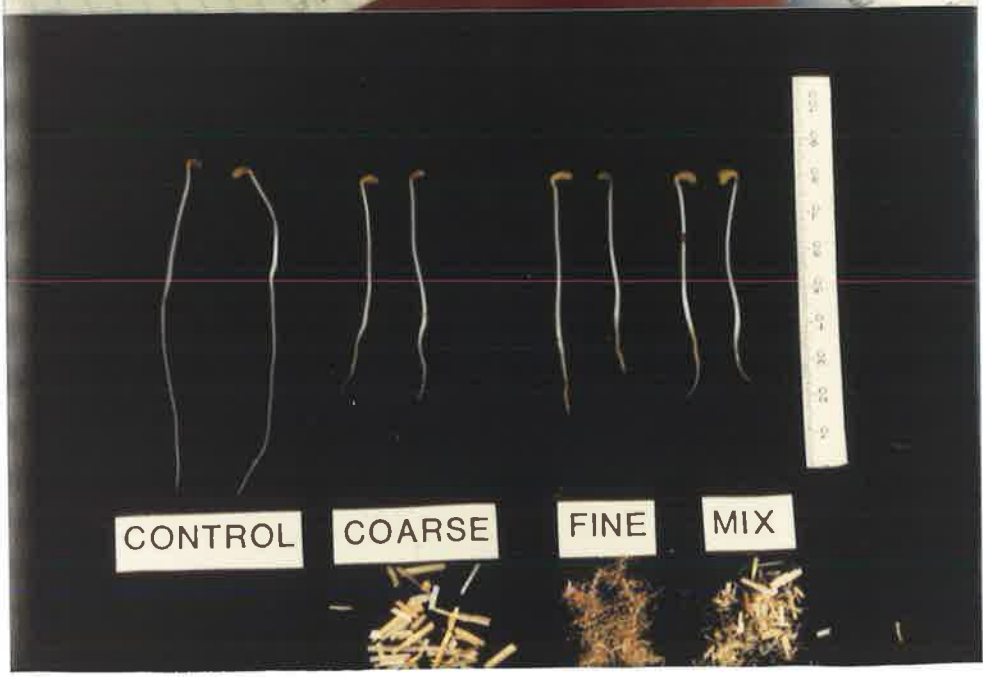
† Non-sterile leachate; †† sterile leachate; * $P < 0.05$; **$P < 0.01$; ns not significant.

Plate 5.1: Bioassay assemblies and techniques

Upper: A bioassay assembly prior to seed germination and seedling growth.

Middle: Retrieval of medic seedlings and measurement of their lengths.

Lower: Representative seedlings from Experiment 5.3.6 (Microbial filtration of leachates and its effect on phytotoxicity), after 5 days. Note the retarded radicle growth of seedlings from the three leachate treatments relative to the control.


CONTROL
COARSE
FINE
MIX

Le Tourneau *et al.* (1956) found that root development of pea seedlings was not affected by sucrose solutions with osmotic potential below 4.5 atm but root growth by wheat seedlings was inhibited by sucrose solutions with osmotic potential of 3 atm.

5.3.7 Leachates applied to seedlings in a soil system (I)

Certain phenolic compounds may be transformed into others by biological or physical means (Horsley 1977; Rice 1984). The phenolic acids, which are probably the most widespread phytotoxins, are partially absorbed in soils (Harris and Kimber 1983) but the degree of bonding depends both upon the chemical state of the compound and upon the composition of the clay mineral fraction of soil. Although there is scant evidence available, soil texture may be important in determining the impact of phytotoxins in soil (Rice 1984) the effect of phenols in soil can be diminished by these compounds being readily leached from the upper layers of the soil. Within soil, phenolic compounds may be subjected to decomposition by microorganisms (Henderson and Farmer 1955; Considine and Patching 1975; Turner and Rice 1975; Black and Dix 1976; Martin and Haider 1979), adsorption by humified organic matter (Wang *et al.* 1971) and clay minerals (Huang *et al.* 1977) and polymerization (Wang *et al.* 1978). These activities preclude long-term accumulation of phytotoxic substances in the soil thereby preventing widespread, acute phytotoxicity to plants. Several different types of microorganisms have the capacity to metabolize phenolic compounds. Turner and Rice (1975) found that bacteria, fungi and actinomycetes were able to grow in soil which had been amended with ferulic acid but without a source of carbon, such as ferulic acid, they were unable to grow.

Because of these physical and biological factors, the activity of straw leachates in soil may be considerably different to that in a sterile bioassay where soil is absent. Consequently the effects of leachate from barley straw residues on medic seedling development were further evaluated in pot culture with soil sampled from the field at Mallala. These results were compared with previous data from bioassays.

Materials and Methods

Cylindrical plastic containers, 170 mm high, with a cross sectional area of 38.5 cm^2 were packed with 450 g of air-dry, sandy-loam soil from Mallala (a full description of this soil is given in Chapter 6 of this thesis). The soil was irrigated with either 200 ml of a leachate prepared from Weeah barley straw (extraction method described in section 5.1) or with rain water. As this volume of liquid exceeded the amount necessary to bring the soil to field capacity, the excess was collected as it drained from the soil and later used in a bioassay. Twenty seeds of Paraggio barrel medic were distributed over the surface of the soil and a further 50 g of air-dry soil was spread over the seeds covering them to a depth of approximately 2 mm. These containers were then kept in the dark at 20°C. After five days the seedlings were washed from the soil and the hypocotyl and radicle lengths of each seedling measured. Treatments were replicated three times and differences between treatment means were compared by analysis of variance.

The solutions which had passed though the soil, together with the original straw leachate and a water control, were assessed by the bioassay system described in section 5.1. The latter two treatments were replicated six times while the first two treatments were replicated three times. A one-way analysis of variance was used to compare mean seedling lengths: however, because the number of replicates varied, it was necessary to compute two LSDs at P=0.05 for the respective comparisons with the control.

Results

The bioassay appeared to be a more sensitive test of phytotoxicity than comparisons of seedling development in a soil system (Table 5.9). Seedlings which were washed from the soil on Day 5 had significantly shorter roots if the soil had been irrigated with leachate from barley straw rather than with water, but the hypocotyls of these young plants were not affected by leachate.

An unidentified factor present in the soil enhanced the phytotoxicity of solutions which had drained through the soil (Table 5.9). On Day 5, root lengths of medics in this treatment were less than half the root length of the control seedlings. Water which had percolated through the soil also developed phytotoxicity by Day 5 of the bioassay. The

leachate, which had not passed through the soil, also reduced radicle extension but was significantly different to the control only on Day 3 and Day 5 and was much less phytotoxic than the leachate recovered from the soil. There was no action taken to eliminate the microbial population from the soil, therefore it is probable that metabolic products from these organisms may have been partly responsible for retarding the growth of seedling roots, similar to the reductions in legume growth associated with straw residues observed by Iswaran and Harris (1968).

TABLE 5.9: Effects of leachate from Weeah barley on seedling elongation of *Medicago truncatula* cv. Paraggio in bioassay and concurrently in a soil system.

Figures in bold are significantly different from the water control in bioassay or indicates significant difference (P<0.05) between treatments in the soil system.

Treatment	Day 3			Day 4			Day 5		
	Hypocotyl	Radicle	Total	Hypocotyl	Radicle	Total	Hypocotyl	Radicle	Total
Bioassay				(mm)					
Water control	25.0	40.0	65.0	38.4	53.8	92.2	47.2	63.0	110.1
Fresh leachate	22.9	**31.6**	**54.5**	37.2	46.1	83.3	47.9	**56.1**	104.0
Water via soil	26.1	36.7	62.8	43.1	49.2	92.3	52.6	**49.4**	102.0
Leachate via soil	24.2	**18.3**	**42.5**	41.9	**26.0**	**67.9**	50.1	**34.4**	**84.5**
Soil in pots									
Soil + water							67.7	52.9	120.6
Soil + leachate							64.7	**45.4**	110.2

5.3.8 Leachates applied to seedlings in a soil system (II)

In the previous experiment leachate from a single variety of barley (Weeah) was applied to one soil sample. The following experiment is an extension of these investigations as an additional barley cultivar was included and two soil samples were compared. Throughout earlier experiments described in this chapter a temperature of 20^{0}C was generally maintained as this is close to optimum for seed germination and seedling emergence of *M. truncatula* (Adem 1977). However, a lower temperature was used in this experiment, more representative of soil temperatures experienced during late autumn in the wheat-sheep zone.

Materials and Methods

Cylindrical plastic containers, 170 mm high, and waith a cross sectional area of 38.5 cm^2 were packed with 650 g of air-dry soil , using either the same soil as in the previous experiment or a sandy loam soil collected approximately two kilometres from the source of the first soil. The containers were irrigated with 140 ml of deionized water in a control or leachate from either Galleon barley or Weeah barley straw. Thirty seeds of Paraggio barrel medic were distributed over the soil surface in each container and the seeds were then covered with 50 g of air-dry soil which covered the seed to a depth of approximately 2 mm. All containers were kept in the dark at 10^{o}C for six days after which seedlings were washed from the soil and seedling lengths measured. There were three replicates of each treatment and a factorial analysis of variance was used to test treatment effects.

Results

The analysis of variance for lengths of seedling radicles is shown below. There were no significant differences between the two soils. There was a small but significant difference between the control and the two straw leachates when mean radicle lengths of seedlings were compared: however, there was no difference in total seedling lengths when the leachate treatments were compared with one another (Table 5.10).

Analysis of variance for radicle lengths of seedlings

Source of variation	DF	Sum of squares	Mean square	F value	Signif. level
Reps	2	4.86	2.43	0.441	ns
Soil	1	2.42	2.42	0.440	ns
Leachate source	2	63.87	31.94	5.803	*
Soil x Leachate source	2	14.29	7.15	1.300	ns
Residual	10	55.03	5.50		
Total	17	140.47	8.26		

The 10^0C temperature slowed seedling growth considerably, for their lengths were less than half those of the seedlings in the previous experiment where a temperature of 20^0C was maintained. The slower metabolism and growth of seedlings may have caused the seedlings to be less responsive to the leachates. These results support the observations made by Steinsiek *et al.* (1982) who concluded that greater phytotoxicity occurs at higher temperatures.

TABLE 5.10: The effects of leachates from Weeah and Galleon barley straws on the elongation of seedlings of *Medicago truncatula* cv. Paraggio in two soils, six days after sowing.

	Soil 1			Soil 2			***Mean***		
Leachate	Hypocotyl	Radicle	Total	Hypocotyl	Radicle	Total	Hypocotyl	Radicle	Total
	Length (mm)								
Control	17.4	27.5	44.9	17.0	24.4	41.4	17.2	26.0	43.2
Weeah	15.1	22.3	37.4	16.5	23.4	39.9	15.8	22.9	38.7
Galleon	16.1	21.6	37.7	17.3	21.4	38.7	16.7	21.5	38.2
Signif. of Diff.							ns	*	ns
LSD (P=0.05)								3.0	

5.4 Discussion and conclusions

The bioassay techniques used in these experiments were simple, rapid and gave results with acceptable repeatability. With this method the seedlings grew vertically and straight which allowed easy measurement of seedling hypocotyl and radicle lengths.

An aspect of these studies was the short duration over which seedling growth was observed viz. three to six days after seed imbibition commenced. This period is the most relevant for study as seedlings are most vulnerable to phytotoxic leachates during the initial stages of seedling growth, particularly where the seedlings are in close proximity to phytotoxins, that is, in the top layer of soil where most of the medic seed germinates. In the field, leachates from plant residues are most likely to be concentrated in the top few centimetres of soil, especially when rain falls in light showers (Kloot and Boyce 1982). When radicles extend into lower layers of soil, where phytotoxins are less concentrated, further radicle growth may proceed at more normal rates regardless of phytotoxin concentrations at the soil surface. Considerably more research needs to be conducted on the mechanism of plant uptake of phytotoxins, the translocation of these compounds, the effects of prolonged exposure and the responses of older plants (McCalla and Norstadt 1974; Rice 1984).

The seedlings in the bioassays were moistened only once by leachate whereas repeated application of leachate would more closely simulate the situation in the field where sequential rains each cause leaching from straw residues. However, there is little data in the literature describing the rates of detoxification of phytotoxins in soil under field conditions (Cochran *et al.* 1977), therefore the significance of repeated exposure of plants to fresh phytotoxins is poorly understood.

Comparison of leachates obtained by spraying a straw sample with water (section 5.3.4) or covering straw with water for an extended period (sections 5.3.2 and 5.3.3) indicated that leachates from both methods have similar effectiveness in reducing root growth of medic seedlings. Spraying the straw with water is a better simulation of rainfall in the field but is more laborious and time consuming than simply soaking straw in water. Agitation of straw during soaking has no effect on the level of inhibition in bioassay

according to the results of Steinsiek *et al.* (1982). They also found that leachates obtained by either soaking straw or sprinkling straw with water (simulated rainfall) gave similar results in a bioassay conducted at 25°C but at a higher temperature of 35°C the latter method generally caused greater inhibition (Steinsiek *et al.* 1982).

The frequency and intensity of leaching processes have not been critically examined and these would be important aspects for future investigation. The pattern of rainfall may be of paramount importance in the field as the extent to which medic seedlings may be affected by leachates from straw residues depends on the relative rates that phytotoxins are liberated from straw and inactivated in soil. The relative proportions of water and straw chaff (200:8) used in the soaking process to extract phytotoxins were chosen arbitrarily in this work. A rational approach for selection of this ratio has not been previously proposed and choice by other researchers also appears to be arbitrary: for example, Cochran *et al.* (1977) used a 10:1 (water straw) ratio, Shilling *et al.* (1985) used 30:1 and Steinsiek *et al.* (1982) assessed a 300:14 solution.

In view of the dynamic nature of leachate composition found previously by other research groups, it is questionable whether composition is relevant in the present studies and our understanding of the identity of phytotoxic compounds is far from complete (Patrick 1971; Chou and Patrick 1976; Horsley 1977) with greater understanding dependant on future advances in methodology of isolation and identification of compounds (Putnam 1985).

Successful regeneration of annual medic pastures depends on large seed reserves, a moderate germination percentage of seed at any one time and a high level of establishment of seedlings. Environmental factors prejudicial to any of these phases may restrict regeneration but in field situations it may be difficult to detect aberrations in germination, and external influences on establishment may go undetected unless seedlings are carefully monitored. Laboratory methods are often preferable for detailed observation of seed germination.

The germination percentages of seed of a range of annual medic cultivars were slightly reduced by leachate from Galleon barley straw (less than 10 per cent) and germination of *M. truncatula* cv. Paraggio, the chief test cultivar used in the bioassays, was not affected by leachate from straw of a range of cereal cultivars. This evidence strongly

suggests that leachates from cereal straw residues are unlikely to have much impact on the densities of *Medicago truncatula* seedlings in the field. These results also substantiate the observations made in Field Experiment 2 where emergence of Paraggio medic seedlings was unaffected by different concentrations of chaff although presumably there were variable amounts of phytotoxins leached from the chaff and washed into the soil.

The specific effects of phytotoxins on germination depend on the genotype of the seed. Studies have shown that germination of many species vary in response to leachates from residues of specific cereals but the physiological reasons for differences in germination responses are not well understood.

Seed germination of *Medicago littoralis* cv. Harbinger was reduced the most by leachate from straw. This medic had the smallest seeds compared with the other cultivars, therefore greater amounts of phytotoxins may have remained in solution around the seed after imbibition was completed i.e. seed uptake and metabolism of quantities of phytotoxins may have been less during seed imbibition. These results support Waddington (1978) who proposed that toxic products derived from straw residues would probably affect seedling emergence of small seeds more than large ones, because of their larger surface area relative to weight. In the present studies, seed size was confounded with genotype and additional research is warranted in order to clarify the separate effects. The susceptibility of Harbinger is of practical significance as this cultivar is adapted to light-textured soils and short growing seasons of four to five months (Puckridge and Carter 1980), unlike most other cultivars presently available. Harbinger has been sown over extensive areas where environments are suitable. The possibility that allelopathy caused by straw residues may be responsible for reduced plant density in the field should be investigated with Harbinger particularly where the seed are close to the soil surface and hence in close proximity to crop residues. Methods developed in Field Experiment 2, together with bioassays, could be applied in these investigations.

Results from this group of experiments, *in toto*, showed that elongation of seedlings of annual medics, especially growth of young roots, can be retarded when seedlings are in contact with aqueous leachates from cereal straw residues. The observation made here, that root growth of young seedlings is especially sensitive to phytotoxins but seed germination is

comparatively insensitive, is in agreement with conclusions of several other researchers including Guenzi and McCalla (1966a), Patrick (1971), Shilling *et al.* (1985) and Barnes and Putnam (1983, 1986). Retarded elongation of seedling radicles has significant implications for maintenance of medic plant density in the wheat-sheep zone. At the beginning of the growing season, rainfall distribution is often erratic therefore medic seedlings must frequently withstand substantial soil water deficits and severe competition from grasses and weeds for moisture. Dry periods after the opening rains i.e. a false break, can markedly reduce the seedling population (Puckridge and Carter 1980; Puckridge and French 1983). Consequently, seedlings which can rapidly develop a strong root system capable of utilizing moisture from deep in the soil have a pronounced advantage in the climatic environment typical of the wheat-sheep zone of South Australia. Further research may be warranted in order to investigate the pattern of root development of annual medics in the field when different quantities of straw residue are present on the soil surface. Data from a preliminary experiment suggested that straw had no significant effect on root development of medic seedlings six days after germination commenced in soil (see Table 5.11).

The observations made in experiments 5.3.7 and 5.3.8, where leachate from straw caused a 15 per cent reduction in root length of medic seedlings, are not in agreement with results of this experiment. However, the nature of the solutes surrounding the seedling roots were markedly different in these comparisons. In the latter experiment the soil, which was covered by barley chaff, was irrigated with 20 ml of water each day for 5 days, equivalent to approximately 4 mm of rain per day. Small quantities of phytotoxins leached from the chaff during each irrigation were probably metabolized by microbial action and also physically bound to soil particles.

TABLE 5.11: Root length of *M. truncatula* cv. Paraggio grown in pots of sandy loam soil from Mallala with a range of concentrations of chaffed Galleon barley straw on the soil surface.

Straw concentration (g/m^2)	Root length (mm) Replicate I	II	III	***Mean***
0	62.7	59.8	56.0	59.5
200	62.2	60.4	60.9	61.2
400	58.8	61.0	52.5	57.4
600	59.2	60.4	59.1	59.6
800	58.1	60.7	63.8	60.9
				59.7

In contrast, large volumes of straw leachate were directly applied to soil in the former experiments and these quantities of leachate greatly exceeded the amounts likely to be encountered in the field. Considered in total, the results showed that phytotoxic compounds from straw may reduce early root growth of Paraggio medic by between zero and 15 per cent, but negligible retardation is the most likely outcome in the field. Furthermore, evidence has been obtained from these studies which shows that early weathering of straw in the field leads to a reduction in the phytotoxicity of leachates derived from the straw. Because of this weathering effect on straw, there would be little if any phytotoxicity present in leachates from straw at the time when medics are expected to commence germination viz. from mid-April or later.

In conclusion, these bioassays demonstrated considerable variation in the growth responses of annual medics to leachates, caused by both genetic and environmental factors. It is likely that the bioassay techniques employed here were overly sensitive, detecting effects on seedling growth which are unlikely to be expressed in the more complex

environment which exists in the field. In the field, a proportion of medic seed remains enclosed in pods which may be in various stages of decay. Additional research is needed to determine whether the presence of pod material protects germinating seedlings from the effects of phytotoxins.

Residues from non-cereal crops including field peas and brassica grain crops should be examined for potential harmful effects on regeneration of annual medics†. Common plant species which usually accompany medics in pasture swards, such as barley grass (*Hordeum leporinum*), capeweed (*Arctotheca calendula*), wild turnip (*Brassica tournefortii*), annual ryegrass (*Lolium rigidum*) etc. should also be investigated for allelopathic activity. One common volunteer weed of annual pastures in the wheat-sheep zone, wireweed (*Polygonum aviculare*), has been shown to exude potent chemicals with the ability to retard germination and growth of annual medics (Kloot and Boyce 1982).

Mechanical incorporation of straw into soil immediately prior to the pasture phase in a rotation is not a common practice in the wheat-sheep zone: however, some innovative farmers are currently evaluating this cultural practice and have had favourable results. With this technique a high proportion of medic seed is brought into intimate contact with decaying straw residues in soil, and under moist climatic conditions severe phytotoxic effects could be expressed. In view of this potential harmful effect on medics there is justification for research on these aspects using local plant materials and environmental conditions.

† Such work has been carried out under Australian conditions, although not with medics (Lovett and Jessop 1982).

6

PHYSICAL IMPEDANCE OF EMERGENCE OF MEDIC SEEDLINGS AND ENHANCED PATHOGENESIS CAUSED BY STRAW MULCHES

6 PHYSICAL IMPEDANCE OF EMERGENCE OF MEDIC SEEDLINGS AND ENHANCED PATHOGENESIS CAUSED BY STRAW MULCHES

6.1 Introduction

Mulches of straw, hay or old grass have been used to control weeds over many years in intensive horticultural enterprises (Russell 1973). The use of cereal residues as a mulch has more recently been extended to broadacre farming, by farmer adoption of reduced tillage management systems and trash-farming practices. Weed control has been cited as a major benefit derived from these practices (Steinsiek *et al.* 1982; Barnes and Putnam 1983; Moomaw 1985). Suppression of weeds by mulch is attributed to various chemical and physical factors (Swarbrick 1981; Crutchfield *et al.* 1986).

Physical effects of mulch include lower soil temperatures, which reduces germination and growth of some weed species (Russell 1973). In addition, shading may reduce photosynthesis and weaken seedlings. Seeds of some weeds which may be stimulated to germinate when exposed to light are described as positive photoblastic (Smith 1973), hence their germination is reduced by mulch. Physical impedance by mulch may upset the mechanics of seedling emergence (Chambers 1962; Sedgley and Barley 1963; Arndt 1965a) but this aspect has received little research attention so there is scope for greater understanding of this subject. Taylor (1971), noted that among the deficiencies in our knowledge are the effects of disease on the thrust exerted by seedlings and the lateral restraint necessary for effect maximum vertical thrust through the soil. Chemical effects follow from liberation of phytotoxic compounds from straw after leaching by rain and the development of additional phytotoxins during microbial decomposition of residues (Patrick 1971). A wide range of weed species may be retarded by these compounds (de Almeida 1985; Shilling *et al.* 1985). Bioassay studies have frequently been conducted with little attempt to relate results to the field environment, for example Le Tourneau *et al.* (1956), Iswaran and Harris (1968), Kimber (1973a) consequently interpretation of these data may be difficult and its relevance questionable. However, some researchers have noted the need to consider environmental conditions in the field as important moderating factors when

assessing the implications of laboratory bioassays (Guenzi and McCalla 1962; Greenwood and Kimber 1967; Steinsiek *et al.* 1982). In addition to direct chemical and physical effects that straw mulches may have on plants, seedlings may also be attacked by pathogens which have been stimulated by compounds originating from straw residues (Patrick and Koch 1958; Patrick *et al.* 1963, 1964; Singh and Pandy 1966; Menzies and Gilbert 1967; Doran 1980) and it has been suggested that phytotoxins predispose plants to root rots (Carley and Watson 1967; Wall 1984).

Under typical conditions experienced in the cereal-sheep zone of South Australia, the concentration of straw residues in walker rows frequently ranges between 5000 and 9000 kg/ha. This is composed of residue particles of various sizes with the smaller components derived from parts of the cereal ear, including glumes, rachis and awns. Over summer and autumn the plant residues in the walker rows consolidate and compact due to trampling by livestock and weathering, thereby forming a dense, cohesive mulch.

The impact of straw mulches on a range of weed species has been documented in other reports but it is timely to extend these studies, particularly to evaluate the effects of straw on emergence and establishment of annual medics. Bioassay techniques detailed in Chapter 5 demonstrated that leachates from straw residues had small or negligible effect on the germination percentages of *M. truncatula* seed but the development of young roots could be markedly retarded by these same leachates. Further experiments were needed to determine whether straw residues, applied as a mulch, could affect emergence and subsequent establishment of annual medic seedlings growing in a sandy loam soil. Specific aspects examined in these laboratory experiments included:

1. Phytotoxic responses by medic seedlings during germination and emergence following leaching of a straw mulch
2. The influence of straw residues on the incidence of pathogen attack of young medic seedlings
3. Physical impedance to emerging medic seedlings caused by straw residues in different configurations on the soil surface

6.2 Pot culture methodology

The soil used throughout this series of experiments was obtained from the edge of a field at Mallala where it had not been cropped and was also known to be free of seed of annual medics. After weed residues were scraped from the soil surface, the top 10 cm of soil was collected. The air-dry soil was sieved through a 0.95 mm aperture, square-mesh sieve and then carefully moistened to approximately 17 per cent water. It was then stored in moisture-tight containers until required. The pH, measured with a Hanna HI 8416 pH meter (1: 5 in water) on triplicate samples, was 8.2. The field capacity, determined using the methods of Leeper (1964), was 38 per cent.

This sandy loam soil is light in texture, with good structure (Dahmane 1978) and has been described as a solonized brown soil (Northcote classification Gc2, Gn1) which has free lime in the top soil. Dahmane (1978) with soils of this type found mean total nitrogen in the top 0 to 5 cm to be 0.123 ± 0.031 per cent, on grass-dominant sites in the Mallala district.

Seed of *M. truncatula* cv. Paraggio, graded for size i.e. >1.4 mm and< 1.7 mm (see details in Chapter 5) were used throughout these experiments. Assessment of the germination capacity of the seed indicated that 91.5 per cent of the seed was permeable and viable. Black polypropylene pots, with drainage holes, were 80 mm high with a diameter of 110 mm. Chaffed straw of Galleon barley, described in Chapter 5, was used unless otherwise stated. After all soil had been added to the pots and the medic seeds sown, a pressure equivalent to 15.5 kPa was applied to the soil surface in each pot in order to compact the soil. This pressure was derived from a concrete block which weighed 15 kg. Further details of sowing each experiment are provided in later materials and methods sections. Pots were held in a laboratory where temperature was maintained at 20°C unless noted otherwise.

6.3 Experimental

6.3.1 Effects of a cereal chaff mulch and a plastic mulch on emergence and establishment of seedlings of *Medicago truncatula*

Numerous studies have examined the effects of straw mulch on emergence and growth of a range of species. Unfortunately few of these have endeavoured to partition the fundamental causes of the observed effects e.g. phytotoxicity, physical impedance or temperature modification. Barnes and Putnam (1983, 1986) are an exception as they included mulch treatments which were chemically inert and compared these with mulches of cereal residues. These researchers believed that with this approach they could identify, more precisely, the phytotoxic damage attributable to leachates from straw. However these studies failed to examine a range of concentrations of non-phytotoxic mulch materials, to determine physical effects on emergence.

Evidence from field investigations reported in Chapter 3 demonstrated that straw residues had the capacity to inhibit the process of seedling emergence but the incidence of this was erratic. These deleterious effects were identified as separate from any relationship between levels of hard-seededness in medics and the amounts of straw residues.

The initial experiment undertaken here aimed to quantify the inhibition of emergence of annual medic seedlings when covered by a mulch of cereal chaff. In an attempt to eliminate the phytotoxic influence and identify the level of inhibition related to physical impedance, a further series of treatments was imposed which consisted of plastic mulches. These plastic mulches simulated a range of concentrations of straw residues commonly encountered in the field at the end of summer.

Materials and Methods

Pots were prepared by first placing 300 g of moist soil into each pot and levelling the top of the soil. Forty seeds of *M. truncatula* cv. Paraggio were thoroughly mixed into another 100 g batch of soil which was added to the pots. Finally a further 300 g of soil was added to each pot which ensured that the seeds were buried at least 3 cm deep.

Treatments comprised seven concentrations of chaff mulch as well as three concentrations of plastic mulch and a no-mulch control (refer to Table 6.1) and were replicated four times. The plastic mulch was prepared by cutting a polypropylene, woven sack into random shaped pieces with a maximum dimension of 2 cm. These were macerated in a blender, so the final range of dimensions of the particles resembled that in the barley chaff. Mulches were applied then pressure was exerted to compact the soil and mulch. Photographs of cereal straw mulch and plastic mulch can be compared in Plate 6.1. All pots were watered with 50 ml of rainwater immediately after sowing and with 20 ml of rainwater the following day. Seedling emergence above the mulch layer was recorded 5, 6 and 8 days after commencement and seedling mortality was recorded on Day 13 and 21. Development of seedling necrosis had diminished substantially by Day 21 therefore no further score for establishment was undertaken.

Results

Concentrations of chaff mulch of 300 g/m^2 or less did not reduce final emergence of medic seedlings nor did any concentration of plastic mulch significantly reduce emergence when compared with the mulch-free control 8 days after sowing (Table 6.1). Pots with 800 g/m^2 of chaff mulch were destructively sampled on Day 8 to examine the state of seedling development below the mulch. Seedlings were found to be in various stages of decomposition therefore the total number of seedlings present could not be ascertained. A mulch concentration of 800 g/m^2, either of chaff mulch or plastic, caused a retardation in the rate of seedling emergence due in all probability to the greater apparent depth of the seed. The emerging seedlings experienced mechanical impedance for a longer duration in these cases. On Day 13 there was a trend of increased seedling mortality as the concentration of straw mulch increased (Table 6.1), but by Day 21 there was a large degree of variability within treatments, consequently treatment means were not significantly different although the same trend as noted on Day 13 persisted. The mortality of seedlings, as tabulated, occurred after seedling emergence above the mulch. Lesions on the hypocotyl, associated with seedling mortality, were observed at the mulch level. Trends in medic density at emergence on Day 8 and at establishment on Day 21 were curvilinear as

shown in Figure 6.1: however, the range of straw mulch concentrations was limited here and further experiments to define the effects of mulch more completely, are described in Section 6.3.3 which follows. Seedling establishment was significantly retarded when concentrations of chaff mulch exceeded 200 g/m^2 but seedling establishment in the plastic mulch treatments was not significantly different to the no-mulch control.

TABLE 6.1: Effects of straw and plastic mulch on the emergence, mortality and establishment of *Medicago truncatula* cv. Paraggio in sandy-loam soil in pots.

Mulch concentration (g/m^2)	Emergence (#/pot)			Mortality (% of emerged)		Establishment (#/pot)
	Day 5	Day 6	Day 8	Day 13	Day 21	Day 21
Straw						
0	30.8	31.5	31.8	1.1† (6.0)§	12.8† (21.0)§	26.3
50	29.3	31.0	32.0	0.4 (3.5)	11.6 (20.0)	27.8
100	30.5	31.8	32.0	5.7 (13.8)	14.1 (22.1)	26.3
200	26.0	28.0	29.0	12.7 (20.9)	24.7 (29.8)	21.8
300	25.8	29.5	30.0	21.6 (27.7)	36.3 (37.1)	19.0
400	17.3	22.3	22.3	22.1 (28.0)	37.6 (37.8)	13.3
800	2.8	5.0	6.3	-	-	-
Plastic						
200	32.0	32.3	33.0	3.8 (11.2)	6.8 (15.1)	29.3
400	27.3	30.5	30.8	11.6 (19.9)	30.6 (33.6)	20.7
800	21.5	27.0	28.8	6.8 (15.1)	20.8 (27.1)	22.4
LSD (P= 0.05)	4.1	4.2	4.1	13.2	ns	6.8
Signif. of Diff.	**	**	**	*		**

† Retransformed mean after statistical analysis. § Mean with data transformed arcsin$\sqrt{x}$

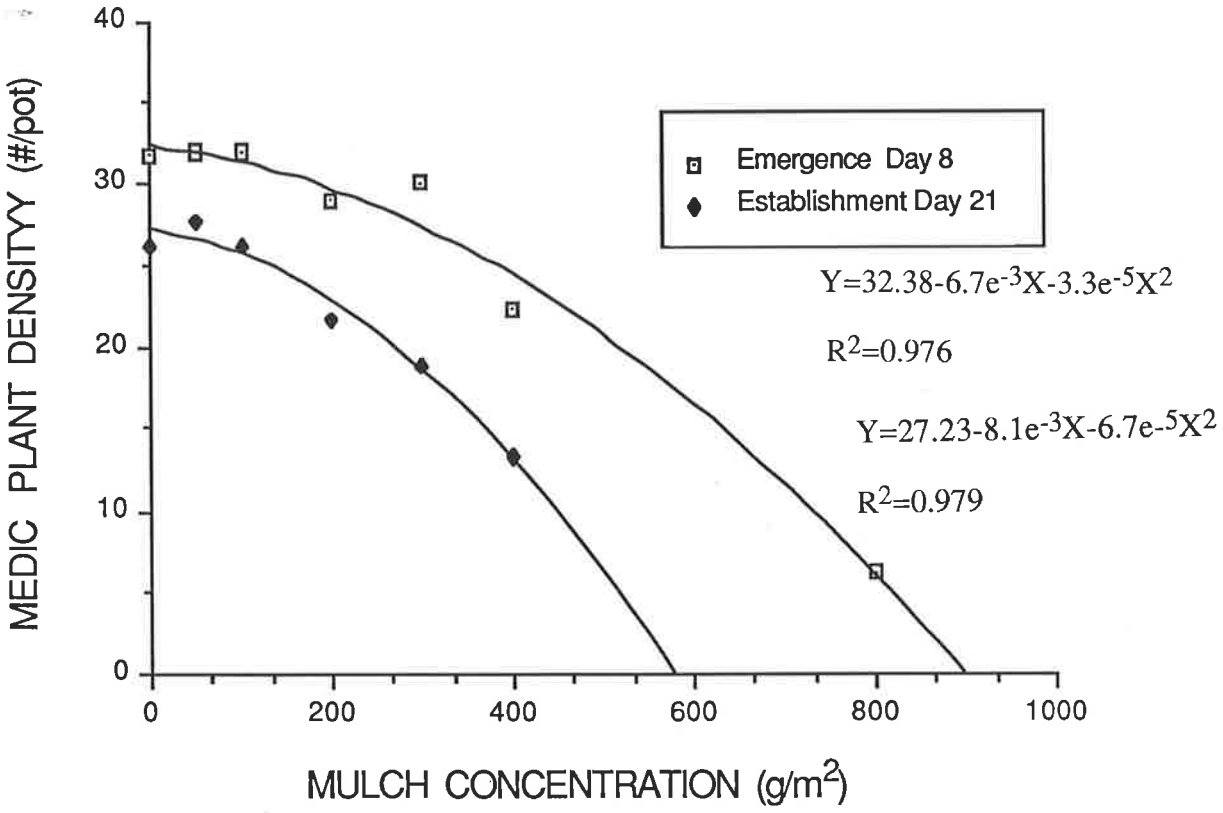


FIGURE 6.1: Emergence and establishment of medic seedlings in pots, related to straw mulch concentrations. Fitted lines derived from regression analyses using a quadratic function.

The plastic mulch treatments were not physically comparable with the straw mulches because the plastic particles failed to adhere together after irrigation with water, therefore they presented little resistance to emerging seedlings. The chaff mulch, in contrast, formed a cohesive layer. This experiment demonstrated that seedlings of annual medic may suffer substantial reductions in emergence if straw residue concentration exceeds 300 g/m^2 but subsequent seedling mortality occurred at high concentrations of chaff so final plant establishment was considerably less than that in a mulch-free comparison. Barnes and Putnam (1983) found that mulches of vermiculite, peat or poplar (not described in detail but presumably wood shavings) failed to reduce emergence of tomato seedlings. Although their results are similar to results from this experiment, the physical configuration and porosity of these mulch materials make it questionable whether the mulches adequately simulated straw residue.

6.3.2 Interaction between seed depth in the soil and chaff mulch concentration

In the previous experiment, high rates of residue mulch were associated with reduced emergence of medic seedlings. This could have been partly due to physical impedance by the mulch i.e. the seedlings were at a greater depth before emerging above the mulch and were therefore under greater stress. Physiological stress such as low temperature, aged seed and deep sowing increases the time between initiation of germination and final emergence and also reduces the vigour of seedlings so the final percentage emergence is reduced. Thus this experiment aims to investigate the effects of stress caused by mulch and compare this with stress induced by increasing depth of sowing.

The preceding experiment also showed that damping-off of medic seedlings was more prevalent where heavy concentrations of straw chaff were present. In order to clarify the contribution that pathogen attack made to establishment failure, a fungicide treatment was included in these studies. The fungicide used was furalaxyl, a systemic acylalanine, highly selective for fungi of the order Peronosporales which includes *Pythium* and *Phytophthora* spp. (Kerkenaar and Kaars Sijpesteijn 1981). This fungicide has been used

to study the etiology of root rot of subterranean clover in dryland pastures and it was found that drenches were neither phytotoxic or stimulatory to seed germination and seedling growth (Greenhalgh and Clarke 1985). *Pythium* is ubiquitous in soils of southern Australia and has frequently been implicated in poor regeneration of annual medic pasture (Andrew 1963; Bretag 1985). However, it is important to determine the variation in susceptibility of different medic species and cultivars to root pathogens. Bretag and Kollmorgen (1986) have evaluated the severity of root rot within a large collection of annual *Medicago* genotypes in soil naturally infested with root attacking pathogens, particularly *Pythium* spp. In their study the medic cultivar Paraggio had a low incidence of attack by pathogens.

Materials and methods

Sowing depth treatments were obtained by first adding a quantity (A) of moist soil to each pot then a further 100 g of soil (B) mixed with forty medic seeds. A third layer of soil (C) was finally added to the pots. The parameters were-

Seed depth	(A)	(B)	(C)
cm	g	g	g
0.5	400	100	50
1.5	300	100	150
2.0	250	100	200
2.5	200	100	250
3.0	150	100	300
3.5	100	100	350

The seed depth treatments were then arranged in factorial combination with 3 concentrations of chaff mulch viz. nil, 100 g/m^2 and 300 g/m^2. An additional group of pots was prepared with all depths of sowing and 300 g/m^2 of chaff mulch and this series was treated with Fongarid® 25 WP† fungicide (active ingredient furalaxyl 250 g/kg) applied in 40 ml of solution (0.25 g a.i. per 1000 ml) after sowing, other pots were irrigated with 40 ml of rain water after sowing. A further 30 ml of rainwater was added to all pots three days later. Seedling emergence was recorded over the first ten days after sowing and seedling mortality was also recorded between Day 7 and Day 16. All

†Manufactured by Ciba-Geigy Australia Ltd, Lane Cove, N.S.W.

treatments were replicated five times and two analyses of variance were used to examine (i) the effects of sowing depth in combination with mulches and (ii) sowing depth in combination with fungicide treatment.

Results

A mulch concentration of 300 g/m^2 (fungicide not applied) significantly ($P<0.05$) reduced seedling density, three and four days after sowing but beyond this time there were no differences between mulch treatments. Furthermore, there was no significant difference in mortality so final establishment was equal (Table 6.2). There was a pronounced effect of reduced seedling density as sowing depth increased but no interaction between mulch concentration and seed depth at any time. Linear regression analysis indicated that percentage seedling emergence declined by approximately 9 per cent as seed depth increased by 1 cm intervals (Figure 6.2). Data obtained by Adem (1977), who also examined the effects of seed depth on seedling emergence of annual medics are also plotted. The seed size he used was comparable to that used in the present studies. The close proximity of Adem's points to the derived line, support the results obtained in this experiment.

Increasing seed depth was generally associated with a trend of greater seedling mortality (Table 6.3). Results obtained from the 2 cm seed depth treatment differed from what was expected in view of the pattern presented by the other five depth treatments; seedling emergence was anomalously low and subsequent mortality of emerged seedlings was unusually high.

Final seedling establishment was a function of both seedling emergence and seedling mortality; both factors in turn were influenced by seed depth, consequently final plant numbers, 16 days after sowing, were markedly reduced as seed depth increased (Table 6.3).

The drenching of pots with fungicide resulted in a significant increase in maximum seedling emergence (measured on Day 10) at all sowing depths (Table 6.4), but the inverse relationship between sowing depth and seedling emergence persisted. The effect of fungicide application on seedling establishment at day 16 was even more pronounced than

the effect on emergence (Table 6.4) and the marked improvement in seedling densities was highly significant ($P<0.01$) at all seed depths.

Eighteen seedlings which had been killed by pathogens were examined by a plant pathologist (Dr P. Pittaway, Waite Institute) to determine the identity of pathogens present. *Pythium ultimum / paroecandrum* which was isolated from seven of the seedlings was the most frequently identified pathogen. *Rhizopus* spp. was less frequent as only three isolates were obtained.

TABLE 6.2: Effects of straw mulch concentration on mortality of emerged seedlings of *Medicago truncatula* cv. Paraggio, and establishment 16 days after sowing. (Mean of all sowing depths)

Straw concentration (g/m^2)	Seedling mortality (% of emerged)				Establishment (%)
	Day 7	Day 10	Day 13	Day 16	Day 16
0	21.7	37.9	45.8	55.4	33.0
100	18.3	36.1	47.5	55.1	33.3
300	25.1	45.6	56.3	67.9	23.0

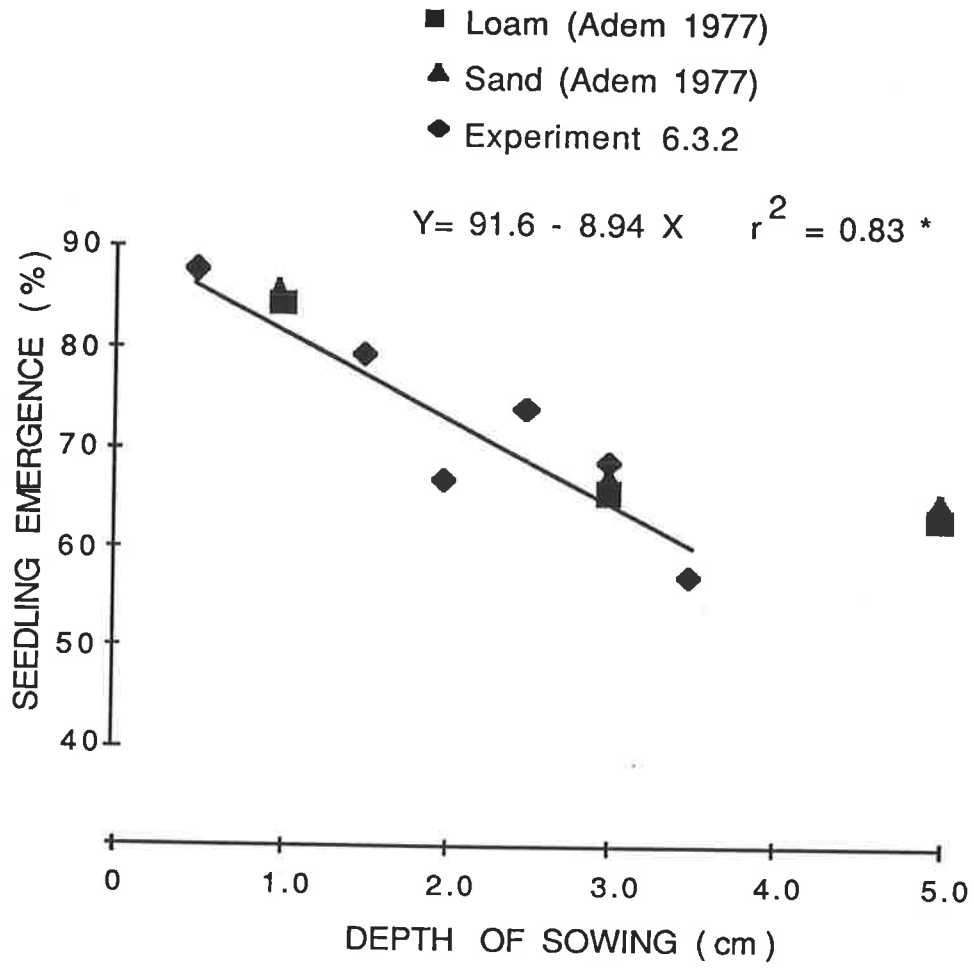


FIGURE 6.2: Medic seedling emergence ten days after sowing seed at six depths including the line fitted by regression. Data points derived from results of Adem (1977), who examined two soil types and their combined effects with depth of sowing, are included here for comparison with the experimental points. Line derived from regression analysis of present experimental data only.

TABLE 6.3: Effects of sowing depth on mortality of emerged seedlings of *Medicago truncatula* cv. Paraggio, and establishment 16 days after sowing.

(Mean of all straw concentration treatments).

Figures in parentheses are means of arcsin√x transformed data

Seed depth (cm)	Seedling mortality (% of emerged)				Establishment (#/pot)
	Day 7	Day 10	Day 13	Day 16	Day 16
0.5	10.6	17.0† (22.2)	23.2† (26.6)	26.0† (29.6)	24.1
1.5	19.5	33.1 (33.8)	40.9 (37.8)	43.6 (40.4)	17.2
2.0	35.3	56.6 (49.5)	66.5 (55.1)	75.4 (61.8)	6.1
2.5	26.2	38.4 (37.2)	43.6 (41.0)	55.9 (50.2)	12.7
3.0	22.7	45.1 (41.1)	60.5 (51.2)	74.4 (60.8)	7.3
3.5	15.9	49.2 (44.3)	64.5 (54.1)	81.3 (66.0)	4.0
Signif. of Diff.	ns	**	**	**	**
LSD (P=0.05)		10.4	10.4	11.3	5.6

† Retransformed mean after statistical analysis

TABLE 6.4: Effects of sowing depth and drenching with fungicide on emergence and establishment of *Medicago truncatula* cv. Paraggio.

All treatments were covered with a 300 g/m^2 chaff mulch

Sowing depth (cm)	Emergence on Day 10 (#/pot)		Establishment on Day 16 (#/pot)	
	No fungicide	Plus fungicide	No fungicide	Plus fungicide
0.5	32.6	34.4	23.5	31.8
1.5	30.4	33.4	15.5	29.5
2.0	23.0	32.2	6.3	23.3
2.5	26.8	30.0	4.5	21.5
3.0	19.8	29.4	2.3	26.3
3.5	18.2	23.4	3.0	16.0
Mean	25.1	30.5	9.2	24.7
	Depth ** LSD(P=0.05) = 2.6		Depth ** LSD(P=0.05) = 5.2	
	Fungicide ** LSD(P=0.05) = 1.7		Fungicide ** LSD(P=0.05) = 7.3	
	Interaction *		Interaction ns	

6.3.3 Mathematical relationships between chaff mulch concentrations and medic seedling establishment and dry matter production

Few attempts have been made to develop detailed relationships between seedling emergence or growth and the concentrations of overlying crop residues but previous studies have had serious limitations because of their experimental designs.

Waddington (1978) showed that seedling emergence of lucerne was inversely related to the amount of rape straw incorporated into soil and a single high rate of wheat straw also inhibited emergence but there was no effect on the leaf area of individual plants which established. This reduction in seedling density was attributed to phytotoxicity of plant residues and in this instance may have been especially severe as unweathered straw was incorporated into the soil.

Barnes and Putnam (1983) obtained different quantities of rye residues by applying herbicides to growing plants of four different ages. Emergence of yellow foxtail and lettuce seedlings was reduced as the age of the rye at killing time increased. The authors claimed that there was a positive correlation between residue biomass and age at time of killing but data which described the quantities of rye residue were not presented in the paper. A further anomaly in the paper was a failure to include in the text, data from control treatments although these existed.

An attempt to develop mathematical relationships between biomass of weeds and the concentration of straw mulch on the soil surface was made by de Almeida (1985) but there are two serious deficiencies in the experimental procedures he used. Mulches of different plant species were used in a single analysis also the equation fitted to the data appeared to be a poor fit and more appropriate functions should have been evaluated. Recently, Crutchfield *et al.* (1986) showed that both plant densities and dry matter yields of weeds declined linearly as the concentration of wheat-straw mulch increased up to 6.8 t/ha. This data was obtained from two field sites over two years but the weed population, which consisted of at least four species, was considered as a single entity. His use of only five mulch treatments may have contributed to this outcome for examination of his data suggests that curvilinear functions may have described the relationship more accurately.

Future investigations to examine the responses of seedlings to variable quantities of mulch should incorporate the following features:

1. The mulch should be on the soil surface and not mixed in to the soil

2. A wide range of mulch concentrations should be examined so that the data provides a strong indication of the most appropriate function to fit and the amounts of residue material are representative of concentrations encountered in the field.

3. Observations should be continued over a long period as establishment may take considerably longer than emergence *per se*, and measurements of seedling mortality should be taken.

4. Attempts should be made to minimize the variation in environmental factors differentially affecting treatments such as soil water potential, temperature, etc.

5. Growth of individual plant species should be assessed rather than a collection of species considered as a single entity.

These criteria were adopted in the design of the following experiments reported below. The experimental objectives were to quantify the levels of medic seedling emergence, establishment and growth in response to different concentrations of mulch and to fit these data to mathematical functions.

Materials and methods

Eleven concentrations of chaff mulch, as shown in Figure 6.3, were applied to pots to which the following sequence of soil had previously been added; 250 g of soil, 100 g of soil mixed with 40 medic seeds, then 200 g of soil. This gave a nominal sowing depth of approximately 2 cm. Another set of pots was prepared to examine the effects of deep sowing, using the sequence, 100 g of soil, 100 g of soil mixed with 40 medic seeds and

finally 350 g of soil, but with only four concentrations of chaff mulch viz. O, 400, 600 and 800 g/m^2. All pots received 60 ml of water immediately after sowing then 20 ml each day for the next 5 days to maintain them at field capacity. The pots were initially located in a laboratory where temperature was maintained at 20°C but after six days they were transferred to a glasshouse. Seedling emergence was recorded until 10 days after sowing and also the numbers of healthy plants per pot were recorded for 17 days. Finally the seedlings in each pot were cut at soil level and values of mean dry weight per seedling determined after drying the tops in a forced-draught dehydrator at 80°C for 24 hours.

Results

When a third order polynomial model was used to fit the data, a strong relationship was found to exist between the maximum seedling emergence measured ten days after sowing and the concentration of chaff mulch (Fig. 6.3a). Some 96 per cent of the variation in seedling emergence was explained by straw concentration in the regression analysis. There was little change in emergence until the mulch concentration exceeded 200 g/m^2 thereafter emergence declined at a relatively steady rate as mulch concentration increased to 600 g/m^2 beyond which the changes in emergence were less. Indeed at these high mulch concentrations seedling emergence was less than 10 per cent of the control.

Values of dry weight per seedling progressively declined as the chaff mulch concentration increased, diminishing more rapidly at higher mulch concentrations and significant regression analysis was again obtained (Fig. 6.3b).

Seedling data from the deep-sown treatments were compared with those from from shallow-sown treatments with corresponding mulch quantities. Analysis of variance indicated that there was no significant difference, caused by sowing depth, in the density of emerged seedlings or establishment nor in mortality of seedlings. However, a wider range of sowing depths could be expected to produce significant differences as was shown in Figure 6.2

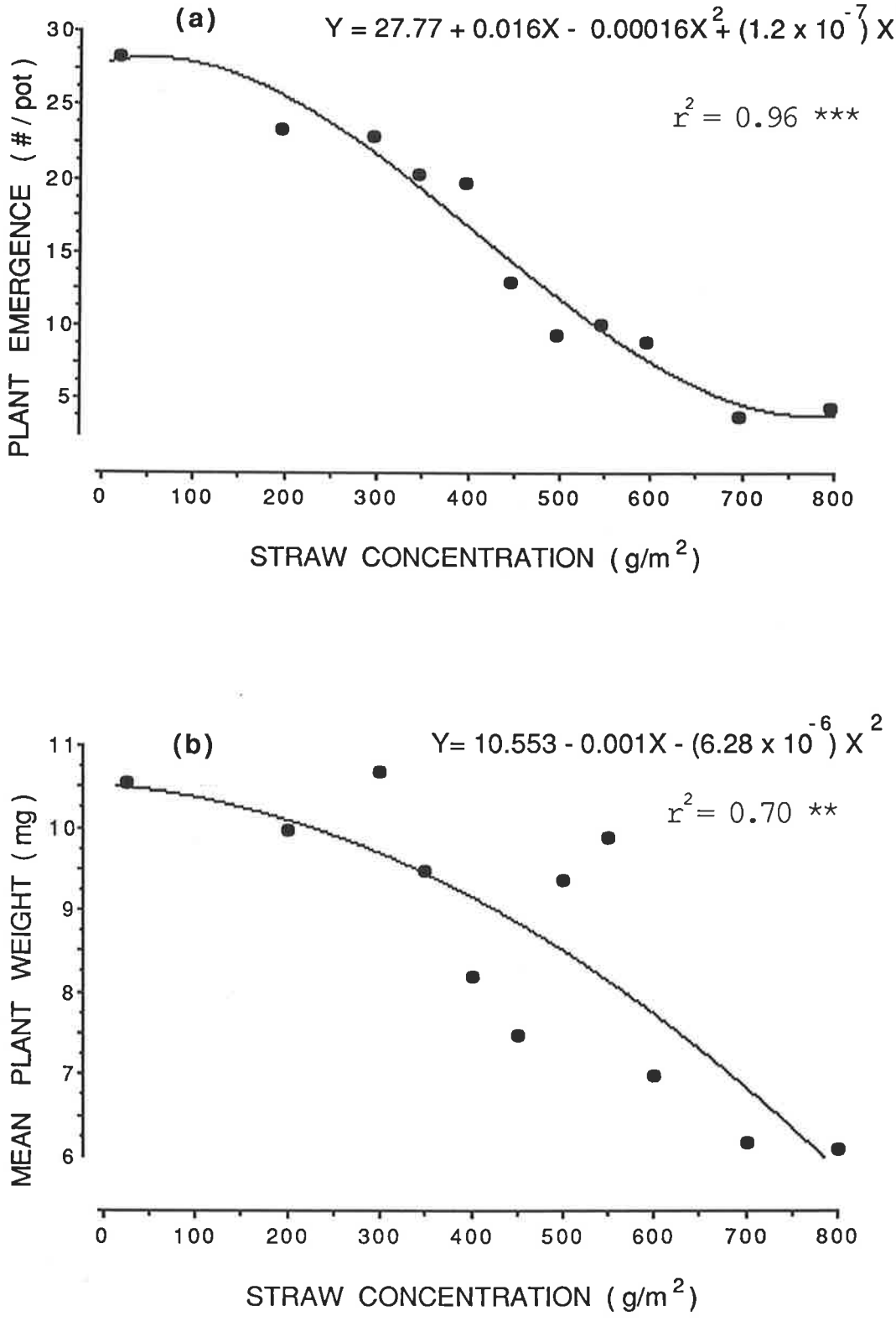


FIGURE 6.3: (a) Emergence of medic seedlings related to straw concentration, ten days after sowing seed

(b) Mean dry weights of seedling tops related to straw concentration, 17 days after sowing seed

6.3.4 Early development patterns of seedling mortality as affected by mulch concentration

Limited observations from section 6.2.1 indicated that a proportion of seedlings began to decompose while in the zone between the soil surface and the lower portions of the mulch. Furthermore, there is evidence that *Pythium* spp. are active pathogens during establishment stages of seedling development, especially when high quantities of residue are present. It remains to be determined whether seedlings fail to emerge above the soil surface or whether they successfully complete this phase but then succumb to attack by pathogens prior to final emergence above the mulch. Therefore the experiment described below aimed to clarify our knowledge of the progress of pathogen attack on young medic seedlings during emergence phases in soil and through mulch, specifically by observation of emergence and mortality of seedlings of different ages.

Materials and Methods

In this experiment, pots were filled in the following sequence, 250 g of soil, 100 g of soil mixed with 40 medic seeds then 200 g of soil giving a nominal sowing depth of 2 cm. Chaff mulches of nil, 200, 400, and 600 g/m^2, were compared and five replicates were used. Sowing of this set of treatments was undertaken each day for four days (designated S1, S2, S3 and S4). Each pot was irrigated with 60 ml of rainwater on the day of sowing and 30 ml on the following day. Six days after sowing S1, seedling emergence above the straw in all pots was recorded. The following day seedling emergence was again measured and the mulches were carefully teased off the pots to enable an assessment of the seedling emergence above the soil surface and to quantify the incidence of seedling necrosis below the chaff mulch.

Results

The densities of seedlings which emerged and their mortality for each time of sowing, are tabulated in Table 6.5. Chaff mulch concentration had little influence on the numbers of seedlings which emerged above the soil surface, except for the 600 g/m^2 mulch treatment in S1 which appears to be anomalously low when compared with corresponding results in S2, S3 and S4; no explanation can be offered for this

discrepancy. The modest inhibition of emergence above the soil, observed at high mulch concentrations, was most likely caused by pathogen attack of seedlings below ground level.

These results agree with the form of response described in the previous section where seedling emergence above the mulch was marginally reduced by a mulch concentration of 200 g/m^2 but progressive, large reductions were recorded as mulch increased to 400 g/m^2 then to 600 g/m^2. Mortality of seedlings below the mulch was the basic cause of impaired levels of emergence above the mulch. Without more detailed studies it is not possible to conclude whether seedlings were weakened by the straw and thus made more susceptible to pathogens or the pathogen propagule density increased because of nutritional stimulation by straw residues, or both effects occurred simultaneously. Pathogen attack on medic seedlings below the straw could be detected early after emergence above the soil (as early as four days after sowing in S4), which is evidence of the rapidity of the pathogen attack.

TABLE 6.5: Effects of the concentration of chaff mulch (Galleon barley) on emergence and mortality of Paraggio barrel medic sown sequentially at one day intervals on dates shown.

Time of sowing	Straw concentration (g/m^2)	Seedling no. above straw (#/pot)		Seedling no. above soil (#/pot)	Survival above soil (#/pot)	Mortality above soil (%)	
		21.XII	22.XII	22.XII	22.XII	22.XII	
S1 (15.XII)	0	33.8	33.8	33.8	33.2	0.6	0.94†
	200	33.2	33.4	33.4	32.6	0.8	1.09
	400	28.0	28.2	29.2	21.8	7.4	2.72
	600	15.8	16.0	23.8	10.6	13.2	3.69
LSD (P= 0.05)		4.4	4.6	4.3	6.3		0.69
S2 (16.XII)	0	33.0	33.6	33.6	32.4	1.2	1.14
	200	29.8	30.6	31.6	26.6	5.0	2.18
	400	25.0	25.4	31.2	21.8	9.4	3.14
	600	14.8	15.6	28.2	16.2	12.0	3.40
LSD (P= 0.05)		3.0	3.6	2.7	4.6		0.98
S3 (17.XII)	0	29.4	30.4	30.4	30.4	0.0	0.71
	200	28.8	31.2	31.4	30.8	0.6	0.94
	400	20.4	26.0	30.4	26.4	4.0	1.91
	600	7.8	14.2	27.2	16.8	10.0	3.17
LSD (P= 0.05)		5.2	5.7	ns	5.6		0.78
S4 (18.XII)	0	26.8	30.4	30.4	29.2	1.2	1.14
	200	19.6	29.6	31.2	30.4	0.8	0.99
	400	3.8	17.8	28.2	25.0	3.2	1.89
	600	2.2	10.8	27.4	23.4	4.0	2.04
LSD (P= 0.05)		4.1	4.7	ns	ns		0.80

† Arcsine $\sqrt{x}$ transformation

6.3.5 Comparisons of straw, chaff and chemically-inert mulches

A mulch of macerated plastic at a concentration of 800 g/m^2 slowed the rate of seedling emergence but did not significantly reduce final seedling density according to results in section 6.3.1. This combination of effects could be attributed to insufficient physical resistance within the layer to impair normal hypocotyl elongation. These results obtained with a non-cohesive mulch support the observations made by Barnes and Putnam (1983) who also examined non-vegetative mulches. Within straw residues in the fields there is a wide range of particle sizes; entire stems, broken leaf material, fine particles from awns and other components of the ear. The larger particles are frequently anchored to the soil by other residue lying above them while smaller components aggregate and are held together by chemical or physical cohesion. As a whole, therefore, the crop residue may present an immovable barrier to emerging seedlings. The influence that physical impedance may have on seedling emergence warrants further study, especially with particles anchored to the soil so that physical resistance is maintained. Consequently, this aspect of impedance was included in the experiment described below where mulches of barley straw or chaff or a combination of the two were compared with plastic mulch with physical dimensions close to those of dried barley stems. A treatment was included where leachate from barley straw was repeatedly applied to plastic mulch in order to estimate chemical effects on seedlings emanating from barley straw residue.

Materials and methods

Uniform pots were prepared using the sequence, 300 g of soil, 100 g of soil plus 40 medic seeds, and 200 g of soil giving a nominal sowing depth of 2 cm. Seven treatments were imposed in order to compare chemically-inert barriers to seedling emergence with material derived from barley residues.

Treatment (a) Bare soil . No mulch applied

(b) Barley chaff 400 g/m^2.

(c) Barley straw 400 g/m^2 - origin the same as for (b)

The dry barley straws were cut so they fitted across the top of the pots and arranged in parallel order on the soil surface in one layer, then in another layer above at 90^o to the first layer. The straw was anchored in position by a plastic annulus, "L" shaped in cross

section, which rested on the straw inside the top rim of the pot. The annulus was held in position by two rubber bands wrapped around each pot, perpendicular to the circumference.

(d) Barley chaff 200 g/m^2 covered by barley straw 200 g/m^2. After covering the soil surface with a chaff mulch, a single layer of straw was applied with pieces in a parallel configuration and anchored as in (c).

(e) Barley chaff 200 g/m^2 covered by a layer of plastic straws. Plastic drinking straws were cut to length and arranged in a parallel order, as a single layer, on top of the chaff and anchored as in (c).

(f) Plastic straw alone, two layers. The straws in the top layer were arranged at 90^o to the straw in the lower layer and anchored as above.

(g) Plastic straw alone, two layers, irrigated with leachate from barley chaff. The same treatment as (f) but pots were irrigated with a leachate prepared from Galleon barley; 8 g soaked in 200 ml water for 16 hours at 2^{o}C.

Views of the mulches for (c) and (f) are shown in Plate 6.1. Treatments were replicated five times. All pots except treatment (g) were irrigated with 40 ml of rainwater immediately after sowing and were supplemented the following day with 30 ml of rainwater, and a further irrigation with 30 ml of rainwater four days after sowing. On these three occasions, leachate was applied to treatment (g) at equivalent volumes to the other treatments. All pots were irrigated with 30 ml of rainwater six and nine days after sowing.

Seedling densities in the pots were recorded at regular intervals until 11 days after sowing and seedling mortality was measured. The number of dead seedlings per pot was transformed to $\sqrt{(x+1)}$ to correct for kertosis before analysis of variance.

Results

The densities of healthy seedlings over the duration of the experiment are shown in Figure 6.4. Plastic straws alone on the soil surface had no effect on the density of seedlings established 11 days after sowing either when irrigated with rain water or with leachate from barley chaff. This result suggests that physical impedance is not an important factor in determining success of seedling establishment. However, this assertion needs

further testing especially in cases where seed has been sown deeply and emerging seedlings consequently have exhausted energy reserves and diminished emergence force.

Three of the treatments, namely plastic straws in combination with barley chaff, barley straw alone or barley straw in combination with barley chaff reduced seedling numbers relative to the no-mulch control. These effects on seedling density were statistically significant throughout the duration of the experiment. Seedling mortality below the mulch would account for the decline in seedling emergence above the mulch. It is notable that repeated addition of leachate to pots failed to affect seedling emergence but residues of barley straw resulted in considerable reduction in medic density (about 50 per cent). These barley straw residues were a ready source of cellulose, a primary substrate for growth of *Pythium* spp. Both sporangia and oospores of *Pythium* are capable of maximum germination in soil within 1 to 3 hours when stimulated by a substrate (Agnihotri and Vaartaja 1967; Stanghellini 1974) but the concentration of polysaccharides is critical for success.

The chaff mulch treatment (b) was found to be heaving on day 5 due to the upward pressure from the seedlings beneath the mulch so that a disproportionate number of seedlings emerged around the perimeter of the pot rather than through the mulch. The lack of a difference between the seedling density for this treatment and that in the control, must be considered in light of the technical problems experienced.

Seedling mortality, as a percentage of emerged seedlings 11 days after sowing, did not significantly differ between treatments and the mean was 3.2 plants per pot.

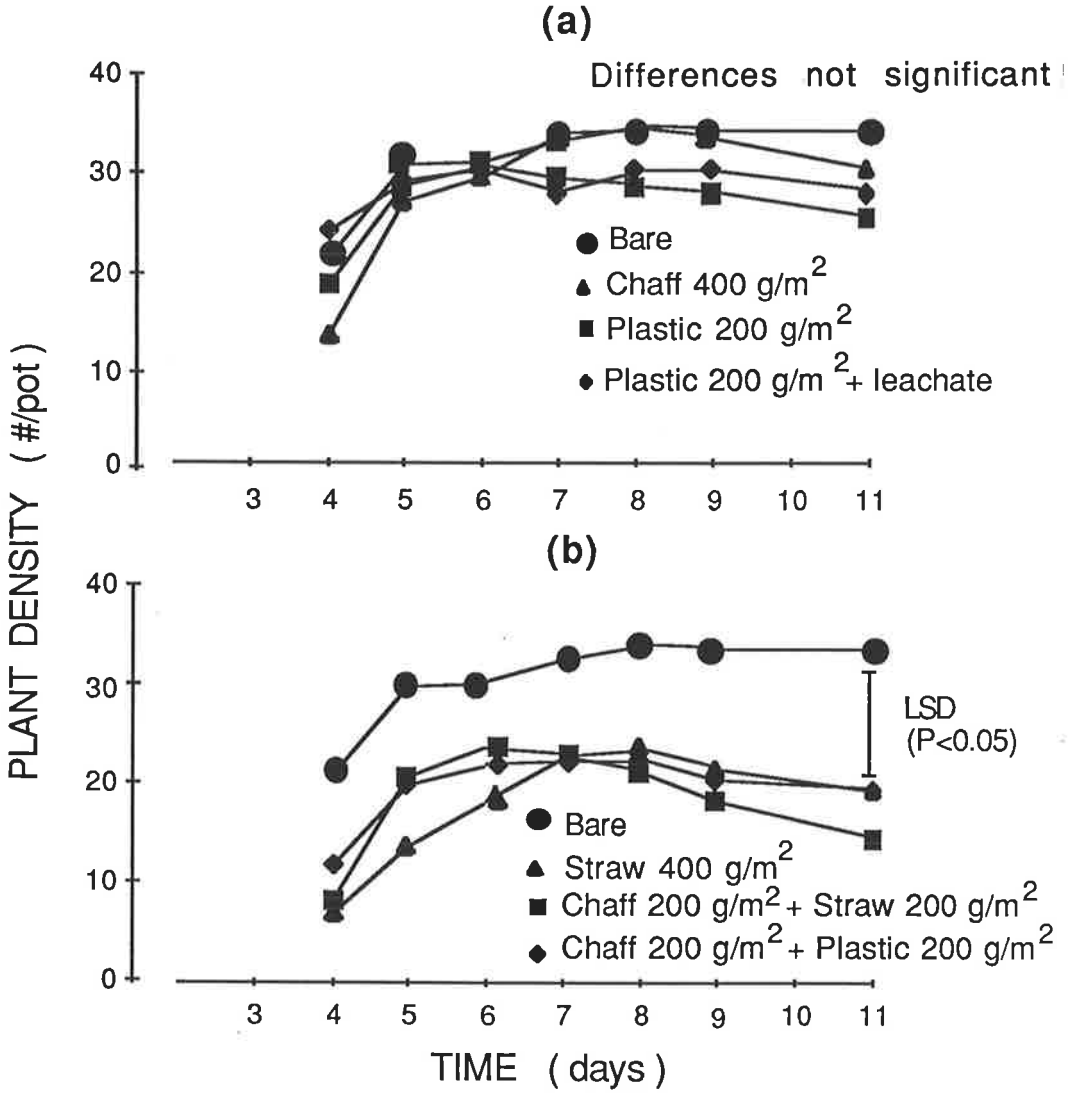


FIGURE 6.4: Medic plant density in each mulch treatment during the experiment.

Results are shown in two parts in order to clarify comparisons.

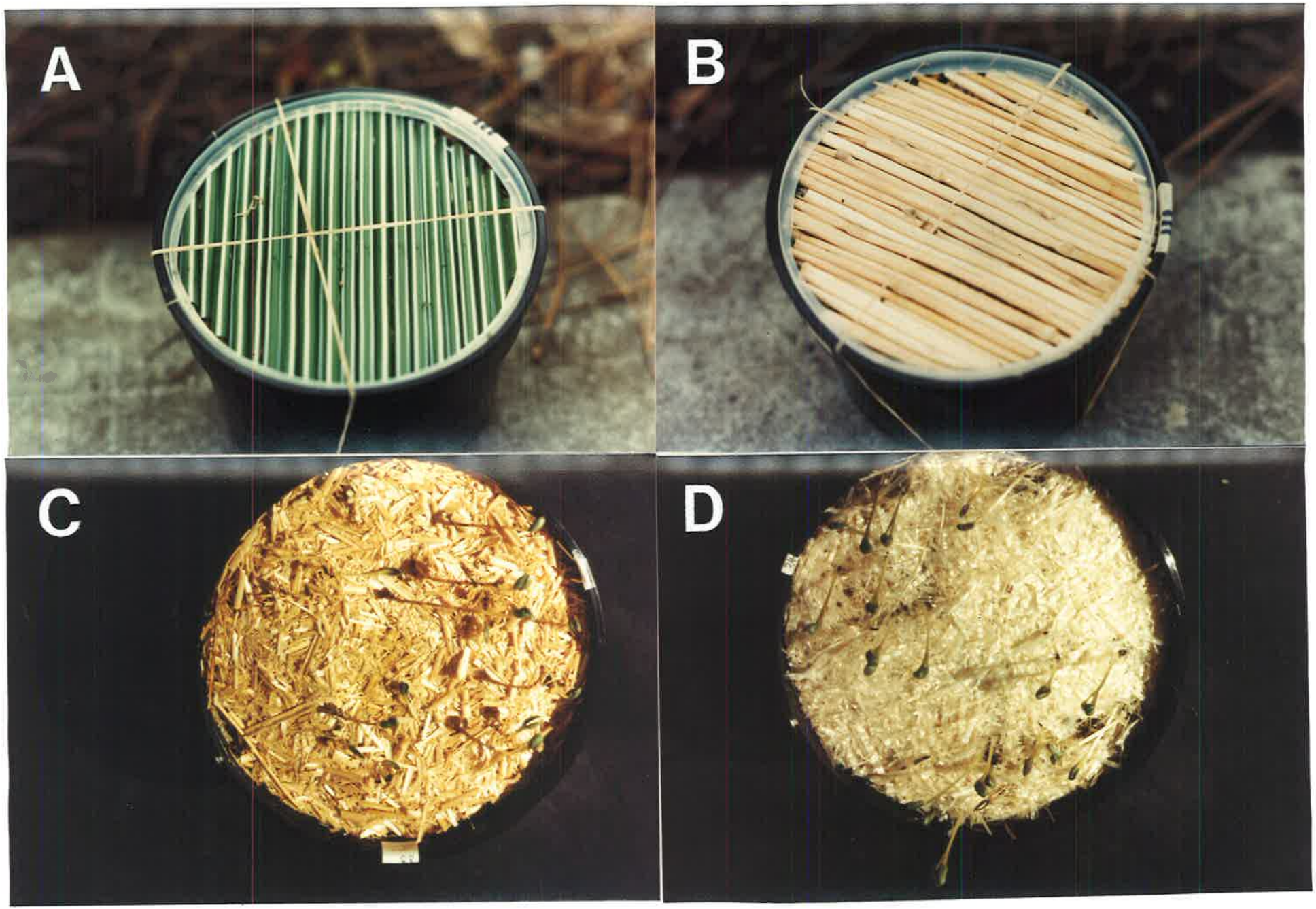
PLATE 6.1: Mulches used in pot studies.

A - A double layer of plastic drinking straws

B - A double layer of mature barley stems

C - Chaff after grinding barley straw

D - Macerated polypropylene


A
B
C
D

6.3.6 Effects of soil sterilization on seedling responses to chaff mulch

Seedling mortality caused by fungal pathogens may be partly responsible for poor seedling emergence above cereal residue mulch but it is not clear whether compounds from the the mulch weaken medic seedlings and predispose them to disease or whether pathogen density increases in response to nutrients supplied by mulch. In the following experiment the confounding effects of pathogens were removed by sterilization of soil. With this treatment it was possible to estimate the combined effects on medic seedlings associated with physical impedance and phytotoxicity. Sterilization of soil has been previously shown to enhance the phytotoxicity from rye residues mixed into soil, as microbial degradation of phytotoxic compounds could not proceed (Barnes and Putnam 1986).

Materials and Methods

Soil was held at 80°C in a forced-draught dehydrator for nine days in order to reduce the number of viable propagules of pathogens responsible for root rot diseases. This sterile soil was then compared with air-dried soil which did not undergo high temperature processing. Seeds had been surface sterilized by immersion in ethanol and mercuric chloride followed by rinses in sterile water, similar to methods of Silsbury *et al.* (1984). A series of eight pots was prepared using each soil, by first adding 400 g of dry soil to each pot. The top surface of the soil was levelled then 40 seeds of medic were sown in a random pattern on the soil, after which they were covered with a further 50 g of dry soil. The soil surface in all pots was covered with a chaff mulch equivalent to 400 g/m^2, but no steps were taken to eliminate microorganisms from the chaff. All water used to irrigate the pots was boiled and then filtered through a millipore filter, with 0.45µm pore size, to avoid microbial contamination. Each pot was irrigated with 40 ml of water when the seeds were sown and a further 40 ml the following day. Four additional irrigations of 30 ml per pot occurred at two day intervals commencing three days after sowing. Seedling emergence and mortality were recorded daily until 14 days after sowing.

Results

Damping-off of seedlings caused a high level of mortality (about 60%) after emergence, where pathogens had not been minimized. Furthermore, in this treatment, total seedling emergence above the chaff mulch was 50 per cent less than that in the sterilized soil (Table 6.6). No plants died in the pots where soil had been sterilized. Neither physical impedance nor phytotoxins leached from chaff affected seedlings in sterile soil as seedling density was close to the theoretical maximum when levels of hard-seededness were taken into account.

TABLE 6.6: Effects of soil sterilization on emergence and establishment of *Medicago truncatula* cv. Paraggio above a straw mulch of 400 g/m^2, 14 days after sowing.

Figures in parentheses are S.E. of the mean

Treatment	Emergence (#/pot)	Establishment (#/pot)	Mortality (% of emerged)
Control soil	16.4 (9.7)	6.6 (3.3)	59.6 (6.9)
Sterilized soil	35.6 (2.2)	35.6 (2.2)	0.0

6.4 Discussion and conclusions

As mulch concentration increased beyond 200 g/m^2 there was a progressive reduction in emergence of medic seedlings above the mulch (found in Experiments 6.3.1, 6.3.3, and 6.3.4). There was little effect of mulch on both medic seed germination in soil and emergence of seedlings through soil but the zone between the soil surface and the top of the mulch was highly hazardous to seedlings. Once seedlings reached this zone some of them were attacked by fungi, identified as chiefly *Pythium* spp. and the proportion of seedlings attacked was a function of the quantity of mulch present. The quantitative relationship was well defined and has been described by a quadratic equation for straw concentrations up to 800 g/m^2. Detailed analysis of seedling response over a wide range of mulch concentrations has not been attempted previously. This analysis is especially important as it advances our knowledge of the fundamental relationship between pathogenic activity and substrate concentration for a saprophytic fungus. Deficiencies in our understanding of this specific aspect of pathogen ecology has recently been cited as surprising (Griffin 1985). The severity of pathogen attack in this zone strongly suggests that propagule density of the pathogen was especially dense and that its growth was stimulated by an ample supply of nutrients provided by straw residue. In contrast, the mortality of seedlings after emergence through the mulch was not as well related to mulch concentration. These effects of soil-borne pathogens could be ameliorated in the laboratory by application of a selective fungicide drench or heat sterilization of soil so near maximum seedling emergence was achievable (Experiments 6.3.2 and 6.3.6).

In retrospect it could have been informative if observations had been made of the incidence of necrotic lesions on roots of seedlings which survived in pots. It would not be unexpected, if in the future, it was found that the incidence of root damage was also related to the amount of straw mulch present. The reduction in dry matter of seedling tops, as mulch concentration increased, was clearly evident in Experiment 6.3.3 which examined this aspect and a reduction in root functioning caused by pathogens could account for these differences. Similar reductions in shoot dry weight have been observed in subterranean

clover when various root pathogens were present (Wong *et al.* 1984) and Barbetti (1984) showed that there was a high negative correlation between severity of root-rot and plant size.

Unweathered barley straw was used as a source of mulch throughout these laboratory experiments and it is highly likely that quantities of phytotoxic compounds were leached from the mulches into soil during irrigation of pots. It would have been desirable to observe whether these leachates affected root morphology or were associated with physiological disfunction of roots which may have predisposed the roots to attack by fungi. Despite this there was no evidence that these phytotoxins directly retarded the emergence of medic seedlings. Leachates from weathered straw would be less phytotoxic and present even less opportunity for harmful effects. The results support the conclusion made in Chapter 5, that it is doubtful whether compounds released from crop residues in the field have any direct impact on emergence and establishment of *M. truncatula* seedlings.

The evidence suggests that physical impedance by a mulch *per se* had little influence on seedling emergence under the specific conditions used here. Further research could be undertaken to evaluate medic responses to mulch under more stressful conditions, for example with deep sowing of seed, waterlogged soil, low temperatures, etc. While these factors all reduce seedling vigour, the relationship between seedling establishment and soil pathogens should remain the foremost priority for investigation.

Pythium spp. were found to be widespread in a survey conducted where medic pastures were grown in rotation with cereal crops (Bretag 1985) and this organism was shown to be an important pathogen which commonly caused root-rot of medics. As a consequence, these pathogens may contribute to the decline in pasture regeneration which has been noted over large areas in the last decade or so. In the field, there may be remarkable differences in the fluctuations of densities of *Pythium* propagules at different sites (Lifshitz and Hancock 1984). The longevity of inoculum is a complex function which may be greatly modified by a variety of soil biotic and abiotic factors (Lumsden *et al.* 1976). Variability in the frequency of *Pythium* would account for the observations made in Field Survey 2 where in some paddocks, reduced medic seedling emergence could be

accounted for by inhibition of hard-seed breakdown alone, but in other cases emergence was less than expected after taking hard-seededness levels into consideration.

Although chemical control of root-rots may be possible in the field it is unlikely to be economical with present materials (Bretag 1985). Medic species vary in their susceptibility to *Pythium* (Andrew 1963) and Bretag and Kollmorgen (1986) demonstrated significant variation in susceptibility between genotypes within a single species. This latter research group have successfully used efficient screening techniques so it is practical to select resistant genotypes of medic as a solution to problems caused by *Pythium*. Selection of plants with such resistance deserves a high priority during future development of new medic cultivars.

7

GENERAL DISCUSSION AND CONCLUSIONS

7 GENERAL DISCUSSION AND CONCLUSIONS

7.1 The relationship between annual medic productivity and the concentration of straw residue in the field

The responses of annual medics to cereal straw residues, although considered important, had not been quantified before the work for this thesis was undertaken. Alternate mechanisms had been postulated to account for the harmful effects of straw residues on annual medics and the ones investigated here were those most likely to account for the field observations. When the relative significance of these alternatives is understood in detail it will be possible to devise rational and effective plans to reduce the deleterious impact of straw residues on medic pastures and to mitigate possible harmful effects of straw residues on medic seed production which contributes to poor seed reserves. This problem of low seed reserves and poor medic pasture production is a widespread over large portions of the cereal-livestock zone of southern Australia (Carter *et al.* 1982).

Residues of cereal straw may be evenly distributed over a particular paddock (frequently the case with wheat straw residues) or they may be partly concentrated in straw walker rows left after grain harvest (commonly found with barley stubble). Large differences in medic plant density are generally visible where dense walker rows are evident. Results from field surveys undertaken over three years during these studies confirmed that cereal straw residues were detrimental to the natural regeneration of annual medics in the field. In most cases, medic plant density was stable throughout the growing season and plant ill-thrift or symptoms of disease where not found during sampling in the field, which suggests that differences in medic plant density were manifested prior to seedling establishment. The reductions in medic plant density, caused by the straw, led to concomitant reductions in dry matter production per unit area. Results from six paddocks studied in Field Survey 1 suggest that the additional straw in the walker rows was responsible for a 16 per cent reduction in dry matter production by medic, on a whole paddock basis (mean of six sites).

Regression analysis of data from field surveys indicated that the model which best described the relationship between medic plant density (or dry matter production) and the

concentration of cereal straw residue was an exponential decay, i.e. small quantities of straw caused the largest changes in medic plant density. The presence of adequate ranges in straw concentration is important for acceptance of the validity of this particular model. Field Experiment 1 deliberately included a complete range of straw concentrations and both relatively high and low values were included. The exponential decay relationship determined in this experiment confirmed the conclusions from the field survey data. Alternative mathematical models were found to be less consistent when all sites were considered together.

In view of the general shape of the response curve determined in these studies, mechanical distribution of the extra straw in walker rows, within the same paddock, would be even more deleterious for pasture productivity. Concentration of the straw in rows affords a good opportunity for mechanical collection of this material and its removal from the field. Concentration in windrows also allows better utilization of crop residues by livestock. This would lead to increases in medic (and subsequent cereal) productivity. The economic advantage of straw removal on subsequent paddock productivity is an important consideration when assessing the full economics of straw utilization, be it for animal consumption or conversion to fuel and has frequently been ignored in past evaluations.

The effects of high concentrations of straw residues in walker rows on medic seed production were examined in Field Survey 1 and Field Survey 3. In the ten paddocks where seed densities were measured there were only three cases where seed production was significantly less within straw walker rows compared with between the rows. This erratic result was influenced by the the variable extent to which medic plants (established between the rows) extended lateral growth into the rows, which in turn depended on the vigour of plant growth. Furthermore, the width of the rows varied and as measurements were made in the centres of the rows this could partly account for the variability in the results. It does not necessarily follow that a reduction in plant density leads to reduced seed set. Adem (1977) found that with a range of medic plant densities between 23 and 14,000 /m^2 there was no difference in seed production. His site, however, was ungrazed throughout his experiment. However, reduced medic density resulting from straw residues

automatically reduces feed availability to livestock during the autumn-winter period when feed is most needed.

Extrapolation of the exponential decay graphs, such as those shown in Figure 3.3 and 3.6, to nil straw on the x axis, provides a prediction of medic plant density in the absence of straw. This value for y is equivalent to the coefficient of *e* in each relevant equation. Determination of this value for each of the 14 sites and comparison with medic densities between walker rows suggests that there was potential for doubling the plant densities of medic if straw was removed entirely. Substantial increases in dry matter production, which is most important through the winter months, would accompany these changes in plant density.

There was a marked reduction in the density of broadleaf weeds within walker rows, compared with densities between rows (see Table 3.1 for example) so there would have been increased competition between broadleaf weeds and medic plants at low straw concentrations. If weeds had been controlled, for example with selective herbicides, the medic response to straw removal would have been expected to be greater than that observed here.

7.2 Changes in hard-seededness of medic influenced by straw residues

These studies support the hypothesis that the major mechanism which links medic plant density with straw residue concentration is retarded breakdown of hard-seededness during summer and autumn. Results from Field Experiments 1 and 3, and from Field Surveys 2 and 4 substantiate this claim. The evidence from present studies validates the proposal of Quinlivan and Millington (1962) that vegetation cover is a major cause of differences in hard seed content and this data supports the findings of Burton (1964): however, the research reported here is more comprehensive.

Van Doren and Allmaras (1978) discussed the theoretical and empirical principles related to diurnal temperature fluctuations in soil and the influences that straw residues may have through their reflectance of thermal radiation.

"For random distribution of cylindrical materials, the following equation holds fairly well:"

$$1 - MFRAC = \exp(-0.000168 \times MWT / DENS \times DIAM)$$

MFRAC=fraction of the soil surface covered by crop residue

MWT = residue dry weight (tons/ha)

DENS=specific dry density of residue (g/cm^3) and

DIAM=diameter of cylindrical residue pieces (m)

According to empirical data presented by these authors, the relationship for wheat straw is

$$1 - MFRAC = 1.17e^{-0.59(MWT)}$$

Van Doren and Allmaras (1978) considered that theoretical determination of temperature changes in soil is precluded because of the layered characteristics of thermal conductivity when vegetation residues are present and the uncertainty in determining net radiation: however, they provided a semi-empirical computation of average temperature difference based on the effect of reflectance of soil and residue on heat flow -

$$\Delta T = T_S - T_R = K (T_I - T_{MIN}) \times [MFRAC \times (P_R - P_S)]$$

T_S = average temperature of bare soil (^0C)

T_R = average temperature of residue covered soil (^0C)

K = constant which accounts for energy sinks other than soil heating

T_I = mean monthly air temperature (^0C)

T_{MIN} = lowest mean monthly air temperature when ΔT is assumed to be zero (^0C)

P_R = reflectivity coefficient of residue

P_S = reflectivity coefficient of soil

In summary, these equations show that the reduction in soil temperature is linearly related to the degree that residue covers the soil (MFRAC), which in turn is exponentially related

to the residue mass per unit area. Cornish (1982) in his discussion on the effects of dead plant litter on radiation transmission to the soil surface also presented an exponential function relating the two. These theoretical considerations strongly support the conclusions from present experimental work.

A feature of these studies was the determination of patterns of breakdown of hard-seededness in natural populations of medic seed sampled from paddocks. Previous research studies concerned with such changes have frequently involved freshly grown seed, for example Hagon (1974), McComb and Andrews (1974), which is not normally representative of seed in paddocks. The importance of this is exemplified by results of Field Experiment 3 where recently-grown seed was compared with seed sampled from soil reserves in a paddock. Although genotypic differences are confounded in this comparison, the prior thermal conditioning of the seed caused marked differences in subsequent germination behavior in the laboratory. Hard seed of cv. Hannaford, which had experienced a range of thermal regimes during the field experiment, had relatively even germination percentages in subsequent laboratory tests; in comparison Jemalong seed had markedly different germination percentages. The variation between cultivars is consistent with the explanation that the hard seed of Jemalong was "conditioned" by mild temperatures in the field which failed to totally break the hard-seededness but stimulated the seed to become permeable when it experienced further thermal fluctuations in the laboratory. The Hannaford seed was either not conditioned at all or was uniformly conditioned (during the years it had existed within the soil reserve); most likely the latter. These results suggest that the long term patterns of germination of these two seed groups would be quite different in the field. There is a pressing need for additional studies of on-farm patterns of change in seed permeability, assessment of germination, emergence, seedling establishment and persistence, under the range of conditions found in normal farming situations.

7.3 Allelopathic effects from cereal straw residues

In south-eastern Australia, annual pasture legumes are important species grown in rotation with cereal crops. In spite of this there have been no previous investigations of

possible allelopathic effects of cereal straw residues on production of annual medics or subterranean clover. Lovett (1982) has drawn attention to this deficiency in contemporary research. The recent laboratory experiments conducted in these studies at the Waite Institute showed that phytotoxic compounds could be leached out of fresh, unweathered cereal straw and that these allelopathic chemicals primarily affected elongation of the radicles of medic seedlings but had insignificant effect on growth of plant tops. Bioassay results were dependent on both the genotype of the straw from which leachate was obtained, and on the genotype of *Medicago* in contact with the leachate. It is not possible, at present, to propose a fundamental reason which accounts for the variable reaction between medic genotypes.

The limitations inherent in bioassay methods must be recognized, for actual behavior of plants in the field are likely to be markedly different to their performance in a bioassay. The need for caution in interpreting bioassay results has been reiterated throughout the scientific literature concerned with allelopathy, for example Guenzi and McCalla (1962) and Fisher (1977). Bioassays are necessary in order to limit the large number of variables which could be involved and they frequently intensify the effects of factors which otherwise might be difficult to isolate.

Evidence was obtained which confirmed that microbial activity attenuates these allelopathic effects. When leachates from straw were added to soil in which young medic seedlings were growing, the reductions in root elongation were minor after 5 to 6 days. Root length of medic seedlings grown in pots of soil was unaffected when the seedlings emerged through different quantities of chaffed-straw mulch. Probable routes of phytotoxin deactivation have been discussed in Chapter 5.

The conclusion drawn from the studies on leachates is that allelopathic effects are not a major cause of poor productivity of annual medic in the field. An aspect which could be examined in greater detail in future, is the possible interaction between repeated application of leachates and intermittent periods of drought and how combinations of these two factors affect establishment, survival and productivity of annual medics. Retarded root development of plants caused by phytotoxins could be expected to reduce the ability of plants to withstand severe water deficits in soil.

7.4 Enhanced pathogenesis associated with straw residues

In many previous studies of allelopathic effects of straw in the field, reductions in plant density have been attributed to phytotoxicity but the validity of this conclusion is open to question because no assessments were made of pathogen activity. It is likely that in some cases effects from pathogens were confounded with responses to phytotoxins. The laboratory studies reported in Chapter 6 provide clear evidence that the quantity of straw overlying the soil was closely related to the level of plant mortality caused by specific fungal pathogens and hence had a profound influence on medic seedling establishment. When a chaffed-straw mulch of 300 g/m^2 was applied, medic establishment began to decline appreciably and at a concentrations of 400 g/m^2 and beyond there were marked reductions in establishment due to attack by *Pythium* fungi.

The shape of this response curve is distinctly different to the functions which were fitted to survey data. This disparity exists largely because effects of hard-seededness were manifested in the field where seedling densities changed rapidly at low straw concentrations (below 300 g/m^2). Intact pieces of straw caused similar effects to the chaffed straw in pots but a plastic mulch did not; leachate from straw also failed to elicite any seedling response. This is consistent with the interpretation that the straw residues provided a ready source of nutrients for the fungi which permitted extensive hyphal development, consequently there was considerable threat to seedlings in this environment.

No attempts were made here to ascertain whether leachate from the straw weakened the medic seedlings and hence predisposed them to attack from pathogens. Future research should include a histological examination of root development where straw leachates or straw mulches have been applied, specifically to determine whether physiological processes within the root are likely to be disrupted. There is a need for additional research to study the incidence of pathogen attack in the field, especially during germination and seedling establishment of medics. The incidence of problems caused by pathogens is likely to be erratic and strongly influenced by edaphic eg. pH, water potential, organic matter content, climatic (temperatures, rainfall) and vegetation conditions (genotype of seedling, presence of mature pasture residues).

There was inconclusive evidence that mulches of straw residue caused physical impedance to emerging medic seedlings. In Experiment 6.3.6 there was no confounding of pathogen effects with physical effects of straw mulch because the soil was sterilized. In this case, seedling emergence was not retarded by a mulch of 400 g/m^2. Furthermore, the seedling densities in Field Experiment 2 were equivalent regardless of mulch concentration up to a maximum of 800 g/m^2. However, localised concentrations of straw may approach 15000 kg/ha in paddocks and at these extremes there is a greater possibility that physical impedance may occur. The studies reported here could have been more conclusive if higher concentrations of residue had been used in the pot experiments and additional studies with straw concentrations exceeding 800 g/m^2 are warranted.

7.5 Future studies warranted

Generally the concentrations of cereal straw residues found in paddocks are too high for optimum promotion of breakdown of hard-seededness in annual medics over summer and autumn. Together with poor seed reserves, this is a major limitation for regeneration of medics and techniques to improve this situation must be urgently developed if agricultural production efficiency is to increase. There is a need to reduce the quantities of straw through changes in farm management and/or the relevant characteristics of medic seed must be alterred by plant breeding; the former proposition offers the greatest potential in the short to medium term and plant breeders will probably be hesitant about selecting for additional traits.

Comparative studies on development of hard-seededness and changes in this characteristic over time, are essential for a fuller understanding of the behavior of medic genotypes especially of new cultivars currently under development including Sephi, Parabinga and Paraggio. Controlled temperature and humidity conditions should be maintained throughout these tests, similar to the assessments conducted in the subterranean clover assessment program based in Western Australia. This work should be integrated with widescale field comparisons so the results from districts with different climates can be related to the standardized assessment. Further research on the fundamental cause of hard-seededness in legumes is essential for development of quicker, quantitative tests of

changes in this trait and would assist in the interpretation of the ecological behavior of annual legumes.

Chapter 3 provided some discussion of alternative means of managing straw residues but burning the stubble was not considered as an option because of several limitations which frequently apply over extensive areas. A ban on burning stubble is usually imposed over summer due to the risk of it escalating into an uncontrolled fire. Burning removes all vegetative cover which leaves the soil prone to wind and water erosion during summer and autumn. Felton *et al.* (1987) suggested that 1000-2000 kg/ha of flattened wheat stubble was desirable for prevention of either wind or water erosion of soil. There is considerable loss of soil nutrients and diminished opportunity for buildup of soil organic matter so plant growth is weaker. Burning destroys medic pods on the soil surface and there is loss of animal fodder.

More research is required on cost-effective methods of mechanical collection and transport of straw also the most advantageous method of utilization of straw, especially by livestock. Some producers have recently considered the possibility of including unweathered straw in rations used to feed sheep during transport to the Middle-east by sea. Other producers are presently investigating the value of collecting straw into several loose stacks within a paddock, mixing some lupin or other high protein grain with the straw, and controlled feeding of this ration to stock. It is desirable that further research be undertaken on the feed value and intake rate of different genotypes of cereal straw residues. These feeding aspects are receiving attention in the Old World, including West Asia and North Africa where new cultivars of cereal are being selected *inter alia* on the basis of increased nutritional value of straw for livestock (Cocks 1987). Australian studies should also investigate the effects that cereal growing conditions, including agronomic practices and disease incidence, have on subsequent grazing and breakdown of straw residues.

8

APPENDICES

Appendix 1: Total monthly rainfall and number of wet days at Mallala in the period 1984-86 and at Two Wells in 1984.

Month	Mallala						Two Wells	
	1984		1985		1986		1984	
	Rain (mm)	Wet days	Rain (mm)	Wet days	Rain (mm)	Wet days	Rain (mm)	Wet days
January	14.0	3	6.8	2	5.2	2	18.2	4
February	2.4	1	0.0	0	7.0	2	2.4	1
March	17.7	5	46.6	2	0.0	0	15.2	5
April	33.6	5	27.4	7	25.2	7	26.5	5
May	27.1	11	27.8	7	15.4	6	43.0	10
June	23.0	6	25.2	7	20.8	9	34.0	8
July	62.6	14	32.4	8	64.8	16	56.0	14
August	61.1	12	57.6	13	84.0	8	76.0	14
September	39.2	12	60.4	9	41.8	8	47.2	14
October	25.4	7	63.0	8	72.0	8	25.2	7
November	31.2	6	16.6	5	24.0	3	27.8	6
December	5.2	2	10.0	5	15.0	5	7.9	5
Total	342.5	84	373.8	73	375.2	74	379.4	93

Appendix 2: Field Experiment 2 - daily rainfall at Mallala over the duration of the experiment.

Date	Rainfall (mm)
June 25	
26	
27	1.8
28	
29	
30	0.8
July 1	5.0
2	10.8
3	2.0
4	6.2
5	
6	
7	13.0
8	3.0
9	8.4
10	3.0
11	0.8
12	
13	
14	
15	
16	4.0
17	2.4
18	0.8
19	
20	
21	
22	0.4
23	2.3
24	
25	
26	
27	
28	1.6
29	1.0
30	

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BIBLIOGRAPHY

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Aase, J.K. and Siddoway, F.H. (1980) Stubble height effects on seasonal microclimate, water balance and plant development of no-till winter wheat. *Agric. Meteorol.* **21**: 1-20.

Adem, L. (1977) Studies on the ecology and agronomy of annual *Medicago* species. M. Ag. Sc. Thesis, The University of Adelaide. 231p.

Agnihotri, V.P. and Vaataja, O. (1967) Effects of amendments, soil moisture contents and temperatures or germination of *Pythium* sporangia under the influence of soil mycostasis. *Phytopathol.* **57**: 1116 - 1120.

Aitchison, E.M., Murray, P.J. and Rowe, J.B. (1986) Improving the nutritive value of round bales of oat straw by treatment with urea or by supplementation with lupins. *Proc. Aust. Soc. Anim. Prod.* **16**: 123 -126.

Aitken, Y. (1939) The problem of hard seeds in subterranean clover. *Proc. Royal Soc. Vic.* **51**: 187-210.

Allden, W.G. (1980) Integration of animals into dryland farming system. Proc. Int. Cong. Dryland Farming, Adelaide, South Australia. pp. 342-378.

Amor, R.L. (1965) Barrel medic (*Medicago tribuloides* Desr.) in the Australian wheat belt. *J. Aust. Inst. Agric. Sci.* **31**: 25-35.

Amor, R.L., Quigley, P.E. Latta, R.A. and Eales J.W. (1985) Medics for the Victorian Mallee and Wimmera - progress and future needs. Proc. 3rd Aust. Agron. Conf., Hobart, Tasmania. p. 203.

Amor, R.L., Quigley, P.E. Latta, R.A. and Eales J.W. (1986) Evaluation of new aphid resistant annual medics in north-west Victoria. *J. Aust. Inst. Agric. Sci.* **52** : 83 - 86.

Anderson, R.C. and Louchs, O.L. (1966) Osmotic pressure influence in germination tests for antibiosis. *Science* **152**: 771-773.

Andrew, W. D. (1956) Interaction of the moisture and nutritional regimes on hard seed production in Barrel medic (*M. tribuloides* Desr.). Proc. Aust. Plant Nutr. Conf., Melbourne, Victoria. pp. 79-91.

Andrew, W. D. (1958) The influence of climate, soil and management on the regeneration of barrel medic (*M. tribuloides*) in the wheat belt of Australia. Proc. Aust. Agrostol. Conf., Armidale, New South Wales. Paper no.16

Andrew, W.D. (1963) Susceptibility to damping-off in seedlings of *Medicago denticulata* Willd. and *M. minima* (L.)Bert. during the early post-emergence period. *Aust. J. Biol. Sci.* **16**: 281-283.

Armstrong, G.M., Rohrbaugh, L.M., Rice, E.L. and Wender, S.H. (1970) The effect of nitrogen deficiency on the concentration of caffeoylquinic acids and scopolin in tobacco. *Phytochem.* **9**: 945-948.

Army, T.J., Weise, A.F. and Hanks, R.J. (1961) Effects of tillage and chemical weed control practices on soil moisture losses during the fallow period. *Soil Sci. Soc. Am.* **25**: 410-413.

Arndt, W. (1965a) The nature of the mechanical impedence to seedlings by soil surface seals. *Aust. J. Soil Res.* **3**: 45-54.

Arndt, W. (1965b) The impedance of soil seals and the forces of emerging seedlings. *Aust. J. Soil Res.* **3**: 55-68.

Aston, A.R. and Fischer, R.A. (1986) The effect of conventional cultivation, direct drilling and crop residues on soil temperatures during the early growth of wheat at Murrumbateman, New South Wales. *Aust. J. Soil Res.* **24**. 49-60.

Awadhwal, N.K. and Thierstein, G.E. (1985) Soil crust and its impact on crop establishment: a review. *Soil Till. Res.* **5**: 289-302.

Ballard, L.A.T. (1973) Physical barriers to germination. *Seed Sci. Technol.* **1**: 285-303.

Ballard, L.A.T. (1976) Strophiolar water conduction in seeds of the Trifolieae induced by action on the testa at non-strophiolar sites. *Aust. J. Plant Physiol.* **3**: 465-469.

Barbetti, M.J. (1984) Relation between plant size and severity of root rot in subterranean clover. *Aust. J. Exp. Agric. Anim. Husb.* **24**: 126-129.

Barbetti, M.J. (1986) Effect of season, trash and fungicides on fungi associated with subterranean clover. *Aust. J. Exp. Agric.* **26**: 431 - 435.

Barbetti, M.J. and MacNish, G.C. (1983) Root rots of subterranean clover. *J. Agric., W.A.* **1**: 9 - 10.

Barbetti, M.J. Sivasithampovan, K. and Wong D.H. (1986) Root rot of subterranean clover. *Rev. Plant Pathol.* **65**: 287 - 295.

Barley, K.P. (1976) Mechanical resistance of the soil in relation to the growth of roots and emerging shoots. *Agrochimica* **20**: 173-182.

Barnes, J.P. and Putnam, A.R. (1983) Rye residues contribute weed suppression in no-tillage cropping systems. *J. Chem. Ecol.* **9**: 1045-1057.

Barnes, J.P. and Putnam, A.R. (1986). Evidence for allelopathy by residues and aqueous extracts of rye (*Secale cereale*). *Weed Sci.* **34** : 384 - 390.

Behmer, D.E. and McCalla, T.M. (1963) The inhibition of seedling growth by crop residues in soil inoculated with *Penicillium urticae* Bainier. *Plant Soil* **18**: 199-206.

Bell, D.T. (1974) The influence of osmotic pressure in tests for allelopathy. *Trans. Ill. State Acad. Sci.* **67**: 312-317.

Bhalla, P.L. and Slattery, H.D. (1984) Callose deposits make clover seed impermeable to water. *Ann. Bot.* **53**: 125-128.

Bhowmik, P.C. and Doll, J.D. (1982) Corn and soybean response to allelopathic effects of weed and crop residues. *Agron. J.* **74**: 601-606.

Bhowmik, P.C. and Doll, J.D. (1984) Allelopathic effects of annual weed residues on growth and nutrient uptake of corn and soybeans. *Agron. J.* **76**: 383-388.

Black, A.L. (1973) Crop residue, soil water, and soil fertility related to spring wheat production and quality after fallow. *Soil Sci. Soc. Am. Proc.* **37**: 754-758.

Black, J.N. (1955) The influence of depth of sowing and temperature on pre-emergence weight changes in subterranean clover (*Trifolium subterraneum* L.). *Aust. J. Agric. Res.* **6**: 203-211.

Black, J.N. (1956) The influence of seed size and depth of sowing on pre-emergence and early vegetative growth of subterranean clover (*Trifolium subterraneum* L.). *Aust. J. Agric. Res.* **7**: 98-109.

Black, J.N. (1963) The interrelationship of solar radiation and leaf area index in determining the rate of dry matter production of swards of subterranean clover (*Trifolium subterraneum* L.). *Aust. J. Agric. Res.* **14**: 20-38.

Black, R.L.B. and Dix, N.J. (1976) Utilization of ferulic acid by microfungi from litter and soil. *Trans. Brit. Mycol. Soc.* **66**: 313-317.

Blum, U., Dalton, B.R. and Rawlings, J.O. (1984) Effects of ferulic acid and some of its microbial metabolic products on the radicle growth of cucumber. *J. Chem. Ecol.* **10**: 1169-1191.

Blum, U., Dalton, B.R. and Shann, J.R. (1985a) Effects of various mixtures of ferulic acid and some of its microbial metabolic products on cucumber leaf expansion and dry matter in nutrient culture. *J. Chem. Ecol.* **11**: 619-641.

Blum, U., Dalton, B.R. and Shann, J.R. (1985b) Effects of ferulic and p-coumaric acids in nutrient culture of cucumber leaf expansion as influenced by pH. *J. Chem. Ecol.* **11**: 1567-1582.

Bolland, M.D.A. (1985) Effects of phosphorus on seed yields of subterranean clover, serradella and annual medics. *Aust. J. Exp. Agric.* **25**: 595-602.

Bolland, M.D.A. (1986) A laboratory assessment of seed softening patterns of hard seeds of *Trifolium subterraneum* subspp. *subterraneum* and *brachycalycinum* and of annual *Medicago* spp. *J. Aust. Inst. Agric. Sci.* **52**: 91-94.

Bolland, M.D.A. and Collins, W.J. (1987) Persistence of seed produced from single seed crops of subterranean clover on sandy soils near Esperance, Western Australia. *Aust. J. Exp. Agric.* **27**: 81-85.

Borner, H. (1960) Liberation of organic substances from higher plants and their role in the soil sickness problem. *Bot. Rev.* **26**: 393-424.

Bretag, T.W. (1985) Fungi associated with root rots of annual *Medicago* spp. in Australia. *Trans. Br. Mycol. Soc.* **85**: 329-334.

Bretag, T.W. and Kollmorgen, J.F. (1986) Effect of trifluralin, benomyl and metalaxyl on the incidence and severity of root disease in annual *Medicago* spp. and evaluation of cultivars for resistance to root rot. *Aust. J. Exp. Agric.* **26**: 65-70.

Brownlee, H. and Scott, B.J. (1974) Effects of pasture and cereal sowing rates on production of undersown barrel medic and wheat covercrop in western New South Wales.*Aust. J. Exp. Agric. Anim. Husb.* **14**: 224-230.

Burns, J.C. (1982) Integration of grazing with other feed resources in "Nutritional limits to animal production from pastures". Ed. J.B.Hacker. (Comm. Agric. Bur., U.K.) pp. 455-471.

Burton, M.V. (1964) Seedcoat permeability in *Medicago truncatula.* M. Agric. Sc. Thesis, The University of Adelaide. pp. 153.

Butler, J.E. (1983) Dry heat scarification of *Stylosanthes guianensis* seed. *Qld. J. Agric. Anim. Sci.* **40**: 15-18.

Carley, H.E. and Watson, R.D. (1967) Plant phytotoxins as possible predisposing agents to root rots. *Phytopathol.* **67**: 401-404

Carter, E.D. (1974) The potential for increasing cereal and livestock production in Algeria. A report prepared for CIMMYT and the Ministry of Agriculture and Agrarian Reform, Algeria. Waite Agric. Res. Inst. 54 p. plus appendices.

Carter, E.D. (1980) The survival of medic seeds following ingestion of intact pods by sheep. Proc. 1st. Aust. Agron. Conf., Lawes, Queensland. p. 178.

Carter, E.D. (1981) Seed and seedling dynamics of annual pastures in South Australia. Proc. XIV Int. Grassl. Cong., Lexington, Ky., U.S.A. pp. 447-450.

Carter, E.D. (1982) The need for change in making the best use of medics in the cereal-livestock areas of southern Australia. Proc. 2nd Aust. Agron. Conf., Wagga Wagga, N.S.W. p. 180.

Carter, E.D. (1985) Biennial Report of the Waite Agricultural Research Institute 1984-85. p. 78.

Carter, E.D. (1986) Soil core sampling for estimating seed reserves of pasture legumes. Proc. Workshop on tillage systems, rotations, nitrogen and cereal diseases. Waite Agric. Res. Inst. p. N2-N3.

Carter, E.D. (1987) Establishment and natural regeneration of annual pastures. In " Temperate pastures - their production, use and management. Eds. J.L. Wheeler, C.J. Pearson and G.E.Robards. (Aust. Wool Corp./CSIRO, Australia) pp. 35-51.

Carter, E.D. and Challis, S. (1987) Effects of depth of sowing medic seeds on emergence of seedlings. Proc. 4th Aust. Agron. Conf., Melbourne, Victoria. p. 192.

Carter, E.D., Challis, S. and Ridgway, I.G. (1977) The use of heavy solvents for separating seed from soil. Proc. XIII Int. Grassl. Cong., Leipzig, G.D.R. pp. 735-738.

Carter, E.D. and Cochrane, M.J. (1985) The poor subterranean clover status of dairy pastures in the Adelaide Hills. Proc. 3rd. Aust. Agron. Conf., Hobart, Tasmania. p. 217.

Carter, E.D., Wolfe, E.C. and Francis, C.M. (1982) Problems of maintaining pastures in cereal-livestock areas of southern Australia. Proc. 2nd Aust. Agron. Conf., Wagga Wagga, New South Wales. pp. 68-82.

Chambers, S.C. (1962) Bare patch and poor emergence of cereals. III.Crusting of the soil surface. *Western Aust. Dept. Agric. J.* **3**: 453-456.

Chou, C.H. and Patrick, Z.A. (1976) Identification and phytotoxic activity of compounds produced during decomposition of corn and rye residues in soil. *J. Chem. Ecol.* **2**: 369-387.

Cochran, V.L., Elliott, L.F. and Papendick, R.I. (1977) The production of phytotoxins from surface crop residues. *Soil Sci. Soc. Am. J.* **41**: 903-908.

Cochran, V.L., Elliott, L.F. and Papendick, R.I. (1982) Effect of crop residue management and tillage on water use efficiency and yield of winter wheat. *Agron. J.* **74**: 929-932.

Cochrane, V.W. (1948) The role of plant residues in the etiology of root rot. *Phytopathol.* **38**: 185-196.

Cocks, P.S. (1973) The influence of temperature and density on the growth of communities of subterranean clover (*Trifolium subterraneum* L. cv.Mt. Barker). *Aust. J. Agric. Res.* **24**: 479-495.

Cocks, P. S. (1980) Origin and ecology of pasture legumes. *Proc. Int. Cong. Dryland Farming,* Adelaide, South Australia. pp. 282-314.

Cocks, P.S. (1987) Annual report of pasture forage and livestock program, ICARDA, Syria. 288 p.

Cocks, P.S., Mathison, M.J. and Crawford, E.J. (1980) From wild plants to pasture cultivars; annual medics and subterranean clover in southern Australia. In "Advances in legume science". Eds. R.J. Summerfield and A.H.Bunting. (Royal Botanic Gardens, Kew, Ministry of Agriculture, Fisheries and Food). pp. 569-596.

Coile, T.S. (1936) Soil samplers. *Soil Sci.* **42**: 139-142.

Collins, W.J. (1978) The effects of defoliation on inflorescence production, seed yield and hardseededness in swards of subterranean clover. *Aust. J. Agric. Res.* **29**: 789-801.

Collins, W.J. (1981) The effects of length of growing season, with and without defoliation on seed yield and hardseededness in swards of subterranean clover. *Aust. J. Agric. Res.* **32**: 783-792.

Considine, P.J. and Patching, J.W. (1975) Ethylene production by micro-organisms grown on phenolic acids. *Ann. App. Biol.* **81:** 115-119.

Cook, R.J. and Baker, K.F. (1983) The nature and practice of biological control of plant pathogens. (The American Phytopathological Society, St Paul, Minnesota) 539p.

Cook, R.J. and Hoaglund, W.A. (1982) Pythium root rot: A barrier to yield of the Pacific Northwest wheat. Wash. State Univ. Agric. Res. Bull. No.XB0913. p. 20.

Cook, R.J., Sitton, J.W. and Waldher, J.T. (1980) Evidence for *Pythium* as a pathogen of direct-drilled wheat in the Pacific Northwest. *Plant Dis.* **64**: 102-103.

Corcoran, M.R., Geissman, T.A. and Phinney, B.O. (1972) Tannins as gibberellin antagonists. *Plant Physiol.* **49**: 323-330.

Corner, E.J.H. (1951) The leguminous seed. *Phytomorphol.* **1**: 117-150.

Cornish, P.S. (1982) Root development of seedlings of ryegrass (*Lolium perenne* L.) and phalaris (*Phalaris aquatica* L.) sown onto the soil surface. *Aust. J. Agric. Res.* **33**: 665-677.

Cornman, I. (1946) Alteration of mitosin by coumarin and prasorbic acid. *Am. J. Bot.* **33**: 217.

Crutchfield, D.A., Wicks, G.A. and Burnside, O.C. (1986) Effect of winter wheat (*Triticum aestivum*) straw mulch on weed control. *Weed Sci.* **34**: 110-114.

Dagley, S. (1971) Catabolism of aromatic compounds by micro-organisms. *Adv. Microb. Physiol.* **6**: 1-46.

Dahmane, A.B.K. (1978) Nitrogen accretion by annual *Medicago* species in a semi-arid environment. Ph. D. Thesis, The University of Adelaide. pp. 181.

de Almeida, F.S. (1985) Effects of some winter crop mulches on the soil weed infestation. Proc. 1985 Brit. Crop Protec. Conf. -Weeds. pp. 651-659.

Dear, B.S. and Loveland, B. (1985) A survey of seed reserves of subterranean clover pastures on southern tablelands of New South Wales. Proc. 3rd. Aust. Agron. Conf., Hobart, Tasmania. p. 214.

del Moral, R. (1972) On the variability of chlorogenic acid concentration. *Oecologia* **9**: 289-300.

del Moral, R. and Muller, C.H. (1970) The allelopathic effects of *Eucalyptus camaldulensis*. *Am. Mid. Nat.* **83**: 254-282.

del Moral, R., Willis, R.J. and Ashton, D.H. (1978) Suppression of coastal heath vegetation by *Eucalyptus baxterii*. *Aust. J. Bot.* **26**: 203-219.

Denny, G.D., Hogan, J.P. and Lindsay, J.R. (1979) The digestion of barrel medic (*Medicago truncatula*) hay and seed pods by sheep. *Aust. J. Agric. Res.* **30**: 1177-1184.

Donald, C.M. (1951) Competition among pasture plants. I. Intra-specific competition among annual pasture plants. *Aust. J. Agric. Res.* **2**: 355-376.

Donald, C.M. (1959) The production and life span of seed of subterranean clover (*Trifolium subterraneum* L.). *Aust. J. Agric. Res.* **10**: 771-787.

Donnelly, E.D., Watson, J.E. and McGuire, J.A. (1972) Inheritance of hard seed in *Vicia*. *J. Heredity* **63**: 361-365.

Doran, J.W. (1980) Microbial changes associated with residue management with reduced tillage. *J. Soil Sci. Soc. of Am.* **44**: 518-523.

Einhellig, F.A. and Eckrich, P.C. (1984) Interactions of temperature and ferulic acid stress on grain sorghum and soybeans. *J. Chem. Ecol.* **10**: 161-170.

Einhellig, F.A., Shon, M.K. and Rasmussen, J.A. (1982) Synergistic effects of four cinnamic acid compounds on grain sorghum *J. Plant Growth Reg.* **1**: 251-258.

Ekern, P.C. (1950) Raindrop impact as the force initiating soil erosion. *Soil Sci. Soc. Am. Proc.* **15**: 7-10.

Elliott, L.F., Cochran, V.L. and Papendick, R.I. (1981) Wheat residue and nitrogen placement effects on wheat growth in the greenhouse. *Soil Sci.* **131**: 48-52.

Ellis, J.R. and McCalla, T.M. (1973) Effects of patulin and method of application on growth stages of wheat. *App. Microbiol.* **25**: 562-566.

Esau, K. (1977) Anatomy of seed plants 2nd Ed. John Wiley and Sons. pp. 465-466.

Fay, P.K. and Duke, W.B. (1977) An assessment of allelopathic potential in *Avena* germplasm. *Weed Sci.* **25**: 224-228.

Felton, W.L., Freebairn, D.M., Fettell, N.A. and Thomas, J.B. (1987) Crop residue management. In "Tillage: new directions in Australian agriculture". Eds. P.S. Cornish and J.E. Pratley. (Inkata Press, Melbourne and Sydney). pp. 171-193.

Fisher, R. F. (1977) Allelopathic interference among plants. I. Ecological significance. Proc. Fourth North American Forest Biology Workshop, State University of New York. pp. 73-92.

Floss, H.G. (1977) The shikamate pathway. In "Biochemistry of plant phenolics". Eds. T. Swain, J.B. Harborne and C.F. Van Sumere. (Plenum Press, New York and London). pp. 59-89.

Forbes, I. and Wells, M.D. (1968) Hard and soft seededness in blue lupin, *Lupinus augustifolius* L. Inheritance and phenotype classifications. *Crop. Sci.* **8**: 195-197.

French, R.J., Matheson, W.E. and Clarke, A.L. (1968) Soils and agriculture of the northern and Yorke Peninsula regions of South Australia. Special Bull. No. 1.68. 72 p.

Fuerst, E.P. and Putnam, A.R. (1983) Separating the competitive and allelopathic components of interference: Theoretical principles. *J. Chem. Ecol.* **9**: 937-944.

Fukai, S. and Silsbury, J.H. (1976) Responses of subterranean clover communities to temperature. I. Dry matter production and plant morphogenesis. *Aust. J. Plant Physiol.* **3**: 527-543.

Fukai, S. and Silsbury, J.H. (1977) Effect of irradiance and solar radiation on dry matter growth and net CO2 exchange of *Trifolium subterraneum* L. swards at a constant temperature. *Aust. J. Plant Physiol.* **4:** 485-497.

Garland, P.J., Wallens, P.G. and Fenton, G. (1976) Surface stubble retention pays dividends. *Agric. Gaz. N.S.W.* **87**: 17-19.

Garret, S.D. (1970) Pathogenic root-infecting fungi. (Cambridge University Press, London). p. 51.

Gladstones, J.S. (1958) The influence of temperature and humidity in storage on seed viability or hardseededness in the West Australian blue lupin (*Lupinus digitatus* Forsk.) *Aust. J. Agric. Res.* **9:** 171-181.

Gladstones, J.S. (1967) Naturalized subterranean clover strains in Western Australia: A preliminary agronomic examination. *Aust. J. Agric. Res.* **18:** 713-731.

Gladstones, J.S. (1970) Lupins as crop plants (review article) *Field Crop Abstr.* **23**: 123-148.

Glass, A.D.M. (1973) Influence of phenolic acids on ion uptake. I. Inhibition of phosphate uptake. *Plant Physiol.* **51**: 1037-1041.

Glass, A.D.M. (1974a) Influence of phenolic acids upon ion uptake. II. A structure - activity study of the inhibition of phosphate uptake by benzoic acid derivatives. *Bull. Roy. Soc. N.Z.* **12:** 159-164.

Glass, A.D.M. (1974b) Influence of phenolic acids upon ion uptake. III. Inhibition of potassium absorbtion. *J. Exp. Bot.* **25**: 1104-1113.

Glass, A.D.M. (1975) Inhibition of phosphate uptake in barley roots by hydrobenzoic acids. *Phytochem.* **14**: 2127-2130.

Glass, A.D.M. (1976) The allelopathic potential of phenolic acids associated with the rhizosphere of *Pteridium aquilinum. Can. J. Bot.* **54**: 2440-2444.

Glass, A.D.M. and Bohm, B.A. (1971) The uptake of simple phenols by barley roots. *Planta* **100**: 93-105.

Glass, A.D.M. and Dunlop, J. (1974) Influence of phenolic acids on ion uptake. IV. Depolarization of membrane potentials. *Plant Physiol.* **54**: 855-858.

Graham, V.E. and Greenberg, L. (1939) The effects of salicylic aldehyde on the infection of wheat by *Pythium arrhenomanes*, and the destruction of the aldehyde by *Actinomyces erythropolis* and *Penicillium* spp. *Can. J. Res.* **17c**: 52-56.

Greenhalgh, F.C. and Clarke, R.G. (1985) The use of fungicides to study the significance and etiology of root rot in subterranean clover in dryland pastures of Victoria. In "Ecology and management of soil-borne plant pathogens". Eds. C.A. Parker, K.J. Moore, P.T.W. Wong, A.D. Rovira and J.F. Kollmorgen . (American Phytopathological Society, St. Paul, U.S.A.). pp. 234-236.

Greenwood, D.J. (1961) The effect of oxygen concentration on the decomposition of organic materials in soil. *Plant Soil* **14**: 360-376.

Greenwood, D.J. (1968) Measurement of microbial metabolism in soil. In "The ecology of soil bacteria". Eds. T.R.G. Gray and D. Parkinson. (Univ. Toronto Press). pp. 138-157.

Greenwood, E.A.N., Carbon, B.A., Rossiter, R.C. and Beresford, J.D. (1976) The response of defoliated swards of subterranean clover to temperature. *Aust. J. Agric. Res.* **27**: 593-610.

Greenwood, E.A.N. and Kimber, R.W.L. (1967) Inhibition of germination and root growth by extracts of *Bromus mollis* L. straw. *Field Station Record* **6**: 55-60.

Griffin, D.M. (1985) Soil as an envirponment for the growth of root pathogens in "Ecology and management of soil-borne plant pathogens". Eds. C.A. Parker, K.J. Moore, P.T.W. Wong, A.D. Rovira and J.F. Kollmorgen. (American Phytopathological Society, St Paul, U.S.A.). pp. 187-190.

Guenzi, W.D. and McCalla, T.M. (1962) Inhibition of germination and seedling development by crop residues. *Proc. Soil Soc. Am.* **26**: 456-458.

Guenzi,W.D. and McCalla,T.M. (1966a) Phenolic acids in oats, wheat sorghum and corn residues and their phytotoxicity. *Agron. J.* **58**: 303-304.

Guenzi, W.D. and McCalla, T.M. (1966b) Phytotoxic substances extracted from soil. *Soil Sci. Soc. Amer. Proc.* **30**: 214-216.

Guenzi, W.D., McCalla, T.M. and Norstadt, F.A. (1967) Presence and persistence of the phytotoxic substances in wheat, oat, corn and sorghum residues. *Agron. J.* **59**: 163-165.

Hagon, M.W. (1971) The action of temperature fluctuations on hard seeds of subterranean clover. *Aust. J. Exp. Agric. Anim. Husb.* **11**: 440-443.

Hagon, M.W. (1974) Regeneration of annual winter legumes at Tamworth, New South Wales. *Aust. J. Exp. Agric. Anim. Husb.* **14**: 57-64.

Hagon, M.W. and Ballard, L.A.T. (1970) Reversibility of strophiolar permeability to water in seeds of subterranean clover (*Trifolium subterraneum* L.). *Aust. J. Biol. Sci.* **23**: 519-528.

Halsall, D.M. and Gibson, A.H. (1986) Nitrigen fixation associated with straw breakdown. Proc. 8th. Aust. Nitrogen Fixation Conf., Adelaide, Aust. Inst. Agric. Sci. Occasional Publication No. 25. pp. 115-116.

Hamly, D.H. (1932) Softening of the seeds of *Melilotus alba. Bot. Gaz.* **93**: 345-375.

Harborne, J.B. (1977) Variation in and functional significance of phenolic conjugation in plants. In "Biochemistry of plant phenolics". Eds. T. Swain, J.B. Harborne and C.F. Van Sumere. (Plenum Press, New York and London). pp. 457-474.

Harper, S.H.T. and Lynch, J.M. (1981) The kinetics of straw decomposition in relation to its potential to produce the phytotoxin acetic acid. *J. Soil Sci.* **32**: 627-637.

Harper, J.R. and Balke, N.E. (1981) Characterization of the inhibition of K^+ absorption in oat roots by salicylic acid. *Plant Physiol.* **68**: 1349-1353.

Harris, J.R. and Kimber, R.W.L. (1983) Phytotoxins in soil. In "Soils: an Australian viewpoint", Division of Soils, CSIRO. (CSIRO:Melbourne/Academic Press:London). pp. 741-754.

Hartley, R.D. and Harris, P.J. (1981) Phenolic constituents of the cell walls of dicotyledons. *Biochem. Syst. Ecol.* **9**: 189-203.

Hartley, R.D. and Whitehead,D.C. (1985) Phenolic acids in soils and their influence on plant growth and soil microbial processes. In "Soil organic matter and biological activity". Eds. D. Vaughan and R.E. Malcolm. (Martinus Nijhoff/Dr W. Junk Publishers). pp. 109-149.

Henderson, M.E.K. and Farmer, V.C. (1955) Utilization by fungi of p-hydroxybenzaldehyde, ferulic acid, syringaldehyde and vanillin. *J. Gen. Microbiol.* **12**: 37-46.

Hendrix, J.W. (1974) Physiology and biochemistry of growth and reproduction in *Pythium. Proc. Am. Phytopathol. Soc.* **1**: 207-214.

Horricks, J.S. (1969) Influence of rape residue on cereal production. *Can. J. Plant Sci.* **49**: 632-634.

Horsley, S.B. (1977) Allelopathic interference among plants. II. Physiological modes of action. Proc. Fourth North American Forest Biology Workshop, State University of New York. pp. 93-136.

Huang, P.M., Wang, T.S.C., Wang, M.K., Wu, M.H. and Hsu, N.W. (1977) Retention of phenolic acids by non-crystalline hydroxy-aluminium and -iron compounds and clay minerals in soil. *Soil Sci.* **123:** 213-219.

Hyde, E.O.C. (1954) The function of the hilum in some *Papilionaceae* in relation to the ripening of the seed and the permeability of the testa. *Ann. Bot.* **18**: 241-256.

Iswaran, V. and Harris, J.R. (1968) The bacteria of decomposing cereal strain and their effects on plant growth. Ninth Int. Congr. Soil Sci. Trans. III. pp. 501-511.

Ivory, D.A. (1977) Agronomic variation in *Medicago scutellata* and *Medicago orbicularis* in south-eastern Queensland. *Trop. Grasslands* **10**: 165-172.

Jackson, M.G. (1977) Review article : the alkali treatment of straws. *Animal Feed Sci. Tech.* **2**: 105-130.

Jensen, E.H., Frelich, J.R. and Gifford, R.O. (1972) Emergence force of forage seedlings. *Agron. J.* **64**: 635-639.

Jensen, T.E. and Welbourne, F. (1962) The cytological effects of growth inhibitors on excised roots of *Vicia faba and Pisum sativum. Proc. S.D. Acad. Sci.* **41**: 131-136.

Johnstone, G.R. and Barbetti, M.J. (1987) Impact of fungal and viral diseases on pasture. In " Temperate pastures - their production, use and management. Eds. J.L. Wheeler, C.J. Pearson and G.E.Robards. (Aust. Wool Corp./CSIRO, Australia) pp. 235-248.

Kaminsky, R. and Muller, W.H. (1978) A recommendation against the use of alkaline soil extractants in the study of allelopathy. *Plant Soil* **49**: 641-645.

Kassim, G., Scott, D.E., Martin, J.P. and Haider, K. (1981) Stabilization and incorporation into biomass of phenolic and benzoid carbons during biodegradation in soil. *Soil Sci. Soc. Am. J.* **46**: 305-309.

Kerkenaar, A. and Kaars Sijpesteijn, A. (1981) Antifungal activity of Metalaxyl and Furalaxyl. *Pesticide Biochem. Physiol.* **15**: 71-78.

Kimber, R.W.L. (1967) Phytotoxicity from plant residues. I. The influence of rotted wheat straw on seedling growth. *Aust. J. Agric. Res.* **18**: 361-374.

Kimber, R.W.L. (1973a) Phytotoxicity from plant residues. II. The effect of time of rotting of straw from some grasses and legumes on the growth of wheat seedlings. *Plant Soil* **38**: 347-361.

Kimber, R.W.L. (1973b) Phytotoxicity from plant residues. III. The relative effects of toxins and nitrogen immobilization on the germination and growth of wheat. *Plant Soil* **38**: 543-555.

Kirchner, R. and Andrew, W.D. (1971) Effect of various treatments on hardening and softening of seeds in pods of barrel medic. *Aust. J. Exp. Agric. Anim. Husb.* **11**: 536-540.

Kleinig, C.R. (1965) Emergence of *Medicago tribuloides* on moderately acid soils. *Aust. J. Agric. Res.* **16**: 311-319.

Kloot, P.M. and Boyce, K.G. (1982) Allelopathic effects of wireweed (*Polygonum aviculare*). *Aust. Weeds* **1**: 11-14.

Koeppe, D.E., Rohrbaugh, L.M. and Wender, S.H. (1969) The effect of varying U.V. intensities on the concentration of scopolin and caffeoylquinic acids in tobacco. *Phytochem.* **8**: 889-896.

Koeppe, D.E., Southwick, L.M. and Bittell, J.E. (1976) The relationship of tissue chloragenic acid concentrations and leaching of phenolics from sunflowers grown under varing phosphate nutrient conditions. *Can. J. Bot.* **54**: 593-599.

Koeppe, D.E., Rohrbaugh, L.M., Rice, E.L. and Wender, S.H. (1970) The effect of age and chilling temperature on the concentration of scopolin and caffeoylquinic acids in tobacco. *Physiol. Plant.* **23**: 258-266.

Lake, A. and Crawford, E. (1985) Annual medic cultivar recommendations 1985-86. Dept. of Agric., South Australia. Fact Sheet. **20/85**. 4p.

Larson, D.L. and Turner, A.K. (1986) Possible costs for harvesting cereal residues for energy production in Victoria. *J. Aust. Inst. Agri. Sci.* **52**: 45-51.

Leather, G.R. and Einhellig, F.A. (1985) Mechanisms of allelopathic action in bioassay. In "The Chemistry of Allelopathy". Ed. A.C. Thompson. (Amer. Chem. Soc.). pp. 197-205.

Lebedeff, G.A. (1947) Studies on the inheritance of hard seeds in beans. *J. Agric. Res.* **74**: 205-215.

Lee, T.T. (1980) Effects of phenolic substances on metabolism of exogenous indole-3-acetic acid in maize stems. *Physiol. Plant.* **50**: 107-112.

Lee, T.T. and Skoog, F. (1965) Effects of hydrobenzoic acids on indoleacetic acid inactivation by tobacco callus extracts. *Physiol. Plant.* **18**: 577-585.

Leeper, G.W. (1964) Introduction to soil science. 4th. ed. (Melbourne University Press). p. 253.

Lehman, R.H. and Rice, E.L. (1972) Effects of deficiencies of nitrogen, potassium and sulphur on chlorogenic acids and scopolin in sunflower. *Am. Midl. Nat.* **87**: 71-80.

Le Tourneau, D., Failes, G.D. and Heggeness, H.G. (1956) The effect of aqueous extracts of plant tissue on germination of seeds and growth of seedlings. *Weeds* **4**: 363-368.

Levin, D.A. (1976) The chemical defences of plants to pathogens and herbivores. *Ann. Rev. Ecol. Syst.* **7**: 121-159.

Lifshitz, R. and Hancock, J.G. (1984) Environmental influences on the passive survival of *Pythium ultimum* in soil. *Phytopathol.* **74**: 128-132.

Linderman, R.G. and Toussoun, T.A. (1968a) Breakdown in *Thielaviopsis basicola* root rot resistence in cotton by hydrocinnamic (3-phenylproprionic) acid. *Phytopathol.* **58**: 1431-1432.

Linderman, R.G. and Toussoun, T.A. (1968b) Predisposition to *Thielaviopsis* root rot of cotton by phytotoxins from decomposing barley residues. *Phytopathol.* **58**: 1571-1574.

Little, T.M. and Hills, F.J. (1978) Agricultural Experimentation. John Wiley and Sons. 350p.

Lockerman, R.H. and Putnam, A.R. (1979) Evaluation of allellopathic cucumbers (*Cucumis sativus*) as an aid to weed control. *Weed Sci.* **27**: 54-57.

Lockwood, J.L. and Filinow, A.B. (1981) Responses of fungi to nutrient-limiting conditions and to inhibitory substances in natural habitats. *Adv. Microbiol. Ecol.* **5**: 1-61.

Longer, D.E., Lorenz, E.J. and Cothren, J.T. (1986) The influence of seed size on soybean [(*Glycine max* (L.) Merrill] emergence under simulated soil crust conditions. *Field Crop Res.* **14**: 371-375.

Lovett, J.V. (1982) The effects of allelochemicals on crop growth and development. In "Chemical manipulation of crop growth and development". Ed J.S. McLaren. (Butterworths). pp. 93-110.

Lovett, J.V. (1987) Allelopathy in Australia: Bacterial mediation. In "Allelochemicals: Role in agriculture and forestry". Ed. G.R. Waller. (Amer. Chem. Soc., Washington D.C.). pp. 156-174.

Lovett, J.V. and Jessop, R.S. (1982) Effects of residues of crop plants on germination and early growth of wheat. *Aust. J. Agric. Res.* **33**: 909-916.

Lovett, J.V., Hoult, E.H., Jessop, R.S. and Purvis Christine E. (1982) Implications of stubble retention. Proc. 2nd. Aust. Agron. Conf., Wagga Wagga, N.S.W. pp. 101-115.

Lumsden, R.D., Ayers, W.A., Adams, P.B., Dow, R.L., Lewis, J.A., Papavizas, G.C. and Kantzes, J.G. (1976) Ecology and epidemiology of *Pythium* species in field soil. *Phytopathol.* **66:** 1203-1209.

Lynch, J.M. (1977) Phytotoxicity of acetic acid produced in the anaerobic fermentation of wheat straw. *J. App. Bact.* **42**: 81-87.

Lynch, J.M. (1980) Effects of organic acids on the germination of seeds and growth of seedlings. *Plant Cell Envir.* **3**: 255-259

McCalla, T.M. (1943) Microbiological studies of the effect of straw used as a mulch. *Kansas Acad. Sci. Trans.* **46**: 52-56.

McCalla, T.M. and Army, T.J. (1961) Stubble mulch farming. *Adv. Agron.* **13**: 125-196.

McCalla, T.M. and Duley, F.L. (1949) Stubble mulch studies: III. Influence of soil microorganisms and crop residues on the germination, growth and direction of root growth of corn seedlings. *Soil Sci. Soc. Am. Proc.* **14**: 196-199.

McCalla, T.M., Guenzi, W.D. and Norstadt, F.A. (1964) Phytotoxic substances in the stubble mulch system. Trans. 8th Cong. Int. Soil Sci. Soc. III. pp. 933-942.

McCalla, T.M. and Haskins, F.A. (1964) Phytotoxic substances from soil microorganisms and crop residues. *Bacteriol. Rev.* **28**: 181-207.

McCalla, T.M. and Norstadt, F.A. (1974) Toxicity problems in mulch tillage. *Agric. Environ.* **1**: 153-174.

McCann, D.J. and Saddler, H.D.W. (1976) Utilization of cereal straw : a scientific evaluation. *J. Aust. Inst. Agric. Sci.* **42:** 41-47.

McClure, P.R., Gross, H.D. and Jackson, W.A. (1978) Phosphate absorbtion by soybean varieties: the influence of ferulic acid. *Can. J. Bot.* **56**: 764-767.

McComb, J.A. and Andrews, R. (1974) Sequential softening of hard seeds in burrs of annual medics. *Aust. J. Exp. Agric. Anim. Husb.* **14**: 68-75.

McGee, G.C. and Kellock, A.W. (1974) *Fusarium avenaceum*, a seed-borne pathogen of subterranean clover roots. *Aust. J. Agric. Res.* **25**: 549-557.

McKee, G.W., Peiffer, R.A. and Mohsenin, N.N. (1977) Seed coat structure in *Coronilla varia* L. and its relation to hard seed. *Agron. J.* **69**: 53-58.

McLaren, A.D. and Skujins, J. (1968) The physical environment of microorganisms in soil. In "The ecology of soil bacteria". Eds. T.R.G. Grey and D. Parkinson. (University of Toronto Press). pp. 3-24.

McWilliam, J.R., Clements, R.J. and Dowling, P.M. (1970) Some factors influencing the germination and early seedling development of pasture plants. *Aust. J. Agric. Res.* **21**: 19-32.

Machackova, I. and Zmrhal, Z. (1976) Comparison of the effect of some phenolic compounds on wheat coleoptile section growth with their effect on IAA oxidase activity. *Biol. Plant.* **18**: 147-151.

Macindoe, S.L. (1975) History of production of wheat in Australia. In "Wheat and other temperate cereals". Eds. A. Lazenby and E.M. Matheson (Angus and Robertson: Sydney). pp. 99-121.

Marbach, I. and Mayer, A.M. (1974) Permeability of some seed coats to water as related to drying conditions and metabolism of phenolics. *Plant Physiol.* **54**: 817-820.

Martin, J.P. and Haider, K. (1979) Effect of concentration on decomposition of some 14C-labelled phenolic compounds, benzoic acid, glucose, cellulose, wheat straw and *Chlorella* protein in soil. *Soil Sci. Soc. Am. J.* **43**: 917-920.

Martin, J.P., Haider, K. and Kassim, G. (1980) Biodegradation and stabilization after 2 years of specific crop, lignin and polysaccharide carbons in soil. *Soil Sci. Soc. Am. J.* **44**: 1250-1255.

Mayer, A.M. and Poljakoff-Mayber, A. (1975) The germination of seeds. (Pergamon Press, N.Y.). p. 192.

Mengel, K. and Schmeer, H. (1985) Effect of straw, cellulose and lignin on the turnover and availability of labelled ammonium nitrate. *Biol. Fert. Soils.* **1**: 175-181.

Menzies, J.D. and Gilbert, R.G. (1967) Responses of the soil microflora to volatile components in plant residues. *Soil Sci. Soc. Amer. Proc.* **31**: 495-496.

Mercado, B.L. (1979) Introduction to weed science. (Southeast Asia Regional Centre for Graduate Study and Research in Agriculture, Philippines). 292p.

Moje,W. (1966) Organic soil toxins. In "Diagnostic criteria for plants and soil". Ed. H.D. Chapman. University of California, Berkeley. pp. 533-569.

Moomaw, R.S. (1985) An oats (*Avena sativa*) - soybean (*Glycine max*) rotation using ecofarming versus conventional tillage*Weed Sci.*. **33**: 544-550.

Morley, F.H.W. (1958) Effects of strain and temperature on the growth of subterranean clover (*Trifolium subterraneum* L.). *Aust. J. Agric. Res.* **9**: 745-753.

Mulholland, J.G., Combe, J.B., Freer, M. and McManus, W.R. (1976) An evaluation of cereal stubbles for sheep production. *Aust. J. Agric. Res.* **27**: 881-893.

Muller, C.H. (1969) Allelopathy as a factor in ecological process. *Vegetatio* **18**: 348-357.

Nielsen, K.F., Cuddy, T. and Woods, W. (1960) The influence of the extract of some crops and soil residues on germination and growth. *Can. J. Plant Sci.*. **40**: 188-197.

Nielsen, R.L., Stuthman, D.D. and Barnes, D.K. (1961) Interference between oats and alfalfa in mixed seedings. *Agron. J.* **73**: 635-638.

Norstadt, F.A. and McCalla, T.M. (1963) Phytotoxic substances from a species of *Penicillium*. *Science* **140**: 410-411.

Okpala, E.U. (1975) Effect of organic soil amendments on damping-off of lettuce caused by *Corticium praticola*. *Ann. Appl. Biol.* **80**: 155-162.

Parr, J.F. and Papendick, R.I. (1978) Factors affecting the decomposition of crop residues by microorganisms. In "Crop residue management systems". Ed. W.R. Oschwald (ASA Madison, Wisconsin) , special publication no.31. pp. 101-129.

Patrick, Z.A. (1971) Phytotoxic substances associated with the decomposition in soil of plant residues. *Soil. Sci.* **111**: 13-18.

Patrick, Z.A. and Koch, L.W. (1958) Inhibition of respiration, germination and growth by substances arising during the decomposition of certain plant residues in the soil. *Can. J. Bot.* **36**: 621-647.

Patrick, Z.A. and Koch, L.W. (1963) The adverse influence of phytotoxic substances from decomposing plant residues on resistance of tobacco to black root rot. *Can. J. Bot.* **41**: 747-758.

Patrick, Z.A. and Toussoun, T.A. (1965) Plant residues and organic amendments in relation to biological control. In "Ecology of soil-borne plant pathogens". Eds. K.F. Baker and W.D. Snyder. (Univ. Calif. Press, Berkeley). pp. 440-459.

Patrick, Z.A., Toussoun, T.A. and Koch, L.W. (1964) Effect of crop-residue decomposition products on plant roots. *Ann. Rev. Phytopath.* **2**: 267-292.

Patrick, Z.A., Toussoun, T.A. and Snyder, W.C. (1963) Phytotoxic substances in arable soils associated with the decomposition of plant residues. *Phytopathol.* **53:** 152-161.

Patterson, D.T. (1981) Effect of allelopathic chemicals on the growth and physiological response of soybean (*Glycine max*). *Weed Sci.*: 53-59.

Pearce, G.R., Beard, J. and Hilliard, E.P. (1979) Variability in the chemical composition of cereal straws and *in vitro* digestibility with and without sodium hydroxide treatment. *Aust. J. Exp. Agric. Anim. Husb.* **19**: 350-353.

Pearce, G.R., Nwangko, F. and Lee, J.A. (1986) The chemical composition and *in vitro* digestibility of wheat straw in relation to the pattern of growth of the plant. Proc. *Aust. Soc. Anim. Prod.* **16**: 426.

Pedler, A. (1982) The effect of walker straw on the emergence and establishment of medics. Student project, Agronomy Department, Waite Agricultural Research Institute. 23p.

Pfeiffer, N.E. (1934) Morphology of the seed of *Symphoricarpos racemosus* and the relation of fungal invasion of the coat to germination capacity. *Contr. Boyce Thompson Inst. Plant Res.* **6**: 103-122.

Porter, R.H. (1949) Recent developments in seed technology. *Bot. Rev.* **15**: 221-344.

Pratley, J.E. and Cornish, P.S. (1985) Conservation farming - a crop establishment alternative or a whole farm system? Proc. 3rd. Aust. Agron. Conf., Hobart, Tasmania. pp. 95-111.

Puckridge, D.W. and Carter, E.D. (1980) Dryland agriculture in southern Australia and its relationship to other regions - a perspective. Proc. Int. Cong. Dryland Farming, Adelaide, South Australia. pp. 156-190.

Puckridge, D.W. and French, R.J. (1983) The annual legume pasture in cereal-ley farming systems of southern Australia: a review. *Agric. Ecosystems Environ.* **9**: 229-267.

Purvis, C.E., Jessop, R.S. and Lovett, J.V. (1985) Selective regulation of germination and growth of annual weeds by crop residues. *Weed Res.* **25**: 415-421.

Putnam, A.R. and Duke, W.B. (1978) Allelopathy in agroecosystems. *Ann. Rev. Phytopathol.* **16**: 431-451.

Putnam, A.R. (1985) Weed allelopathy. In "Weed Physiology. Vol.I Reproduction and ecophysiology". Ed. S.O. Duke. (CRC Press Inc., Boca Raton, Florida). pp. 131-155.

Putnam, A.R. and Duke, W.B. (1974) Biological suppression of weeds : evidence for allelopathy in accessions of cucumber. *Science* **185**: 370-372.

Quigley, P.E., Carter, E.D. and Knowles, R.C. (1987) Medic seed distribution in soil profiles. Proc. 4th Aust. Agron. Conf., Melbourne, Victoria. p. 193.

Quinlivan, B.J. (1961) The effect of constant and fluctuating temperatures on the permeability of the hard seeds of some legume species. *Aust. J. Agric. Res.* **12**: 1009-1022.

Quinlivan, B.J. (1965a) The influence of the growing season and the following dry season on the hardseededness of subterranean clover in different environments. *Aust. J. Agric. Res.* **16**: 277-291.

Quinlivan, B.J. (1965b) The naturalized and cultivated annual medics of Western Australia. *J. Agric. W. Aust. Series.* **6**: 532-543.

Quinlivan, B.J. (1966) The relationship between temperature fluctuations and the softening of hard seeds of some legume species. *Aust. J. Agric. Res.* **17**: 625-631.

Quinlivan, B.J. (1968a) Seed coat impermeability in the common annual legume pasture species of Western Australia. *Aust. J. Exp. Agric. Anim. Husb.* **8**: 695-701.

Quinlivan, B.J. (1968b) The softening of sand-plain lupin (*Lupinus varius* L.). *Aust. J. Agric. Res.* **19**: 507-515.

Quinlivan, B. J. (1971a) The ecological significance of seed impermeability in the annual legume pastures of southern Australia. Dept. Agric.,W. Aust., Tech. Bulletin No.11. pp. 1-9.

Quinlivan, B.J. (1971b) Seed coat impermeability in legumes. *J. Aust. Inst. Agric. Sci.* **37**: 283-295.

Quinlivan, B.J. (1971c) Embryo dormancy in subterranean clover seeds.II. Its value relative to impermeability in field germination regulation. *Aust. J. Agric. Res.* **22**: 607-614.

Quinlivan, B.J. and Francis, C.M. (1971) The effect of burr burial on the seed of some early maturing subterranean clover cultivars. *Aust. J. Exp. Agric. Anim. Husb.* **11**: 35-38.

Quinlivan, B.J. and Millington, A.J. (1962) The effect of a Mediterranean summer environment on the permeability of hard seeds of subterranean clover. *Aust. J. Agric. Res.* **13**: 377-387.

Quinlivan, B.J. and Nicol, H.I. (1971) Embryo dormancy in subterranean clover seeds. I. Environmental control. *Aust. J. Agric. Res.* **22**: 599-606.

Raleigh, G.J. (1930) Chemical conditions in maturation, dormancy and germination of seeds of *Gymnocladus dioica*. *Bot. Gaz.* (Grawfordville) **89**: 273-294.

Rands, R.D. and Dopp, E. (1938) The influence of certain harmful soil constituents on the severity of *Pythium* root-rot of sugar cane. *J. Agric. Res.* **56**: 53-67.

Rassmussen, J.A. and Einhellig, F.A. (1977) Synergistic inhibitory effects of p-coumaric and ferulic acids on germination and growth of grain sorghum. *J. Chem. Ecol.* **3**: 197-205.

Rasmussen, J.A. and Einhellig, F.A. (1979) Inhibitory effects of combinations of three phenolic acids on grain sorghum germination. *Plant Sci. Letters* **14**: 69-74.

Rice, E.L. (1974) Allelopathy. (Academic Press, New York). 353p.

Rice, E.L. (1979) Allelopathy-an update. *Bot. Rev.* **45**: 15-109.

Rice, E. L. (1984) Allelopathy, 2nd Ed. (Academic Press, New York). 400p.

Rice, E.L. (1985) Allelopathy - an overview. In "Recent advances in phytochemistry. Vol.19. Chemically mediated interaction between plants and other organisms" Eds. G.A. Cooper-Driver, T. Swain and E.E. Conn. (Plenum Press, New York and London). pp. 81-105.

Rice, E.L. and Pancholy, S.K. (1974) Inhibition of nitrification by climax ecosystems. III. Inhibitors other than tannins. *Am. J. Bot.* **61**: 1095-1103.

Robbins, W.W., Craft, A.S. and Raynor, R.N. (1942) Weed control. (McGraw Hill Book Co., New York and London). p. 543.

Rolston, M.P. (1978) Water impermeable seed dormancy. *Bot. Rev.* **44**: 365-396.

Roper, M.M. (1986) N_2 fixation in soils by free-living bacteria utilizing straw for energy. Proc. 8th. Aust. Nitrogen Fixation Conf., Adelaide, Aust. Inst. Agr. Sci. Occassional Publication No. 25. pp. 117-118.

Rowe, J.B. and Ferguson, J. (1986) Lupin grain as a supplement to sheep grazing cereal stubble. *Proc. Aust. Soc. Anim. Prod.* **16**: 343-346.

Russell, E. W. (1973) Soil conditions and plant growth, 10th Ed. (Longman, London and New York). 849p.

Schultz, J. E. (1980) Moisture conservation practices in dryland agriculture. Proc. Int. Cong. Dryland Farming, Adelaide, South Australia. pp. 440-470

Scott, B.J. and Brownlee, H. (1974) Establishment of barrel medic under wheat, oats, barley and linseed in central western New South Wales. *Aust. J. Exp. Agric. Anim. Husb.* **14**: 785-789.

Sedgley, R.H. and Barley, K.P. (1963) The effect of a small axial pressure on the growth of the epicotyl of *Vicia faba* var *minor* Beck. *Aust. J. Biol. Sci.* **16**: 19-27.

Shilling, D.G., Liebl, R.A. and Worsham, A.D. (1985) Rye (*Secale cereale* L.) and wheat (*Triticum aestivum* L.) mulch: The suppression of certain broadleaf weeds and the isolation and identification of phytotoxins. In "The chemistry of allelopathy. Biological interactions among plants". Ed. A.C.Thompson. (American Chemical Society, Washington D.C.). pp. 243-271.

Shindo, H. and Kuwatsuka, S. (1976) Behavior of phenolic substances in the decaying process of plants. IV. Adsorbtion and movement of phenolic acids in soil. *Soil Sci. Plant Nut.* **22**: 23-33.

Silsbury, J.H., Zuill, D. and Brown, P.H. (1984) Effects of temperature on germination, emergence and early seedling growth of swards of Mt. Barker subterranean clover plants grown with and without nitrate. *Aust. J. Agric. Res.* **35**: 539-549.

Singh, R.S. and Pandey, K.R. (1966) Effect of green and mature plant residues and compost on population of *Pythium aphanidermatum* in soil. *Ind. Phytopathol.* **19**: 367-372.

Slattery, H. D. (1982) The inheritance of seed coat impermeability characters in *Trifolium subterraneum* L. Proc. 2nd Aust. Agron. Conf., Wagga Wagga, N.S.W. p. 337.

Slattery, H.D., Atwell, B.J. and Kuo, J. (1982) Relationship between colour, phenolic content and impermeability in the seed coat of various *Trifolium subterraneum* genotypes. *Ann. Bot.* **50**: 373-378.

Smith, H. (1973) Light quality and germination : ecological implications. In "Seed Ecology". Ed. W. Heydecker. (Butterworths, London). pp. 219-231.

Snedecor, G.W. and Cochran, W.G. (1967) Statistical methods, 6th. ed. (The Iowa State University Press). 593p.

Stanghellini, M.E. (1974) Spore germination, growth and survival of *Pythium* in soil. *Proc. Am. Phytopathol. Soc.* **1**: 211-214.

Steinsiek, T.W., Oliver, L.R. and Collins, F.C. (1982) Allelopathic potential of wheat (*Triticum aestivum*) straw on selected weed species. *Weed Sci.* **30**: 495-497.

Stephenson, R.G.A., Pritchard, D.A. and Connell, J.A. (1984) Evaluation of treatments for fortifying round bales of barley stubble using a spear technique. *Proc. Aust. Soc. Anim. Prod.* **15**: 604.

Stewart, G.A., Gartside, G., Gifford, R.M., Nix, H.A., Rawlins, W.H.M. and Siemon, J.R. (1979) The potential for liquid fuels from agriculture and forestry in Australia. (C.S.I.R.O. Australia). pp. 147.

Tomaszewski, M. and Thimann, K.V. (1966) Interactions of phenolic acids, metalic ions and chelating agents on auxin-induced growth. *Plant Physiol.* **41**: 1443-1454.

Toussoun, T.A. (1969) Phytotoxic crop residue decomposition products. In "Nature of the influence of crop residues on fungus induced root diseases". Eds. R.J. Cook and R.D. Watson. pp. 26-27.

Toussoun, T.A. and Patrick, Z.A. (1963) Effects of phytotoxic substances from decomposing plant residues on root rot of bean. *Phytopathol.* **53**: 265-270.

Toussoun, T.A., Patrick, Z.A. and Koch, L.W. (1964) Effect of crop residue decomposition products on plant roots. *Ann. Rev. Phytopathol.* **2**: 267-292.

Toussoun, T.A., Patrick, Z.A. and Snyder, W.C. (1963) Influence of crop residue decomposition products on the germination of *Fusarium solani* f. *phaseoli* chlamydospores in soil. *Nature* **197**: 1314-1316.

Toussoun, T.A., Weinhold, A.R., Linderman, R.G. and Patrick, Z.A. (1968) Nature of phytotoxic substances produced during plant residue decomposition in soil. *Phytopathol.* **58**: 41-45.

Trumble, H.C. (1937) Some factors affecting the germination and growth of herbage plants in South Australia. *J. Dept. Agric. South Aust.* **40**: 779-786.

Tso, T.C., Kasperbauer, M.J. and Sorokin, T.P. (1970) Effect of photoperiod and end-of-day light quality on alkaloids and phenolic compounds of tobacco. *Plant Physiol.* **45**: 330-333.

Turner, J.A. and Rice, E.L. (1975) Microbial decomposition of ferulic acid in soil. *J. Chem. Ecol.* **1**: 41-58.

Unger, P.W. (1978) Straw mulch rate effect on soil water storage and sorghum yield. *Soil Sci. Soc. Am. Proc.* **42**: 486-491.

Van Doren Jnr., D.M. and Allmaras, R.R. (1978) Effect of residue management practices on the soil physical environment, microclimate and plant growth. In "Crop residue management systems". Ed. W.R. Oschwald. (ASA Madison, Wisconsin), special publication no.31. pp. 49-83.

Vercoe, J.E. and Pearce, G.R. (1960) Digestibility of *Medicago tribuloides* (Barrel medic) pods. *J. Aust. Inst. Agric. Sci.* **26**: 67-70.

Waddington, J. (1978) Growth of barley, bromegrass and alfalfa in the greenhouse in soil containing rapeseed and wheat residues. *Can. J. Plant Sci.* **58**: 241-248.

Wall, R.E. (1984) Effects of recently incorporated organic amendments on damping-off of conifer seedlings. *Plant Disease* **68**: 59-60.

Wang, T.S.C., Yang, T.K. and Chuang, T.T. (1967) Soil phenolic acids as plant growth inhibitors. *Soil Sci.* **103**: 239-246.

Wang, T.S.C., Yeh,K.L., Cheng, S.Y. and Yang, T.Z. (1971) Behaviour of soil phenolic acids. In "Biochemical interactions among plants". U.S. National Committee for the International Biological Program. National Academy of Science, Washington D.C. pp. 113-119.

Wang, T.S.C., Li, S.W. and Ferng, Y.L. (1978) Catalytic polymerization of phenolic compounds by clay minerals. *Soil Sci.* **126**: 15-21.

Webber, G.D. (1965) Agriculture in South Australia. The lower north. *J. Dept. Agric. South Australia.* **68**: 282-299.

Webber, G. D., Cocks, P. S. and Jefferies, B. C. (1976) Farming systems in South Australia. (Dept. Agric. and Fisheries, South Aust.) 104p.

Webber, G., Matz, N. and Williams, G. (1977) Ley farming in South Australia. (Dept.Agric.and Fisheries, South Aust.) Bulletin no. 15/77. 19p.

Werker, E. (1980) Seed dormancy as explained by anatomy of embryo envelopes. *Isr. J. Bot.* **29**: 22-44.

Werker, E., Marbach, I. and Mayer, A.M. (1979) Relation between the anatomy of the testa, water permeability and the presence of phenolics in the genus *Pisum*. *Ann. Bot.* **43**: 765-771.

Whitehead, D.C. (1963) Some aspects of the influence of organic matter on soil fertility. *Soil Fert.* **26**: 217-223.

Whitehead, D.C. (1964) Identification of p-hydroxybenzoic, vanillic, p-coumaric and ferulic acids in soils. *Nature* **202**: 417-418.

Whitehead, D.C., Dibb, H. and Hartley, R.D. (1981) Extractant pH and the release of phenolic compounds from soils plant roots and leaf litter. *Soil Biol. Biochem.* **13**: 343-348.

Whittaker, R.H. and Freeny, P.P. (1971) Allelochemics: Chemical interaction between species. *Science* **171**: 757-770.

Williams, C.M.J. and Vallance N.A. (1982) New medic pasture systems to increase production in pasture/cereal rotations in the Victorian Mallee. Proc. 2nd Aust. Agron. Conf., Wagga Wagga. p. 317.

Williams, W.A. (1956) Evaluation of the emergence force exerted by seedlings of small seeded legumes using probit analysis. *Agron. J.* **48**: 273-274.

Williams, W.A. (1963) The emergence force of forage legume seedlings and their response to temperature. *Crop Sci.* **3**: 472-474.

Winter, A.G. (1961) New physiological and biochemical aspects in the interrelationships between higher plants. In "Mechanisms in biological competition". Ed. F.L. Milthorpe (Academic Press, N.Y.) pp. 229-244.

Woodhead, S. (1981) Environmental and biotic factors affecting phenolic content of different cultivars of *Sorghum bicolor*. *J. Chem. Ecol.* **7**: 1035-1047.

Wong, D.H., Barbetti, M.J. and Sivasithamparam, K. (1984) Effects of soil temperature and moisture on the pathogenicity of fungi associated with root rot of subterranean clover. *Aust. J. Agric. Res.* **35**: 675-684.

Zenk, M.H. and Muller, G. (1963) *In vivo* destruction of exogenously applied indolyl-3 acetic acid as influenced by naturally occurring phenolic acids. *Nature* **200**: 761-763.