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INVITED REVIEW

Annual *Medicago*: From a Model Crop Challenged by a Spectrum of Necrotrophic Pathogens to a Model Plant to Explore the Nature of Disease Resistance

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• *Background* Annual *Medicago* spp., including *M. truncatula*, play an important agronomic role in dryland farming regions of the world where they are often an integral component of cropping systems, particularly in regions with a Mediterranean or Mediterranean-type climate where they grow as winter annuals that provide both nitrogen and disease breaks for rotational crops. Necrotrophic foliar and soil-borne pathogens dominate these regions and challenge the productivity of annual *Medicago* and crop legume species.
• *Scope* This review outlines some of the major and/or widespread diseases these necrotrophic pathogens cause on *Medicago* spp. It then explores the potential for using the spectrum of necrotrophic pathogen–host interactions, with annual *Medicago* as the host plant, to better understand and model pathosystems within the diseases caused by nectrotrophic pathogens across forage and grain legume crops.
• *Conclusions* Host resistance clearly offers the best strategy for cost-effective, long-term control of necrotrophic foliar and soil-borne pathogens, particularly as useful resistance to a number of these diseases has been identified. Recently and initially, the annual *M. truncatula* has emerged as a more appropriate and agronomically relevant substitute to *Arabidopsis thaliana* as a model plant for legumes, and is proving an excellent model to understand the mechanisms of resistance both to individual pathogens and more generally to most forage and grain legume necrotrophic pathogens.

Key words: Fungal pathogens, medics, annual *Medicago* species, *Medicago truncatula*, *Phoma medicaginis*, *Aphanomyces euteiches*, *Colletotrichum trifolii*, *Mycosphaerella pinodes*, grain legumes.

INTRODUCTION

Annual *Medicago* spp. play an important agronomic role in dryland farming regions of the world (Walsh *et al*., 2001) where they are often an integral component of cropping systems, particularly in regions with a Mediterranean-type climate (Piano and Francis, 1992) where they grow as winter annuals (Sheaffer and Lake, 1997). Some annual *Medicago* spp. have distinct advantages, such as high levels of hard seededness, that make them attractive over other annual forage legume species such as *Trifolium subterraneum* in many areas.

Annual *Medicago* spp. provide nitrogen for rotational crops (Zhu *et al*., 1998; Sheaffer *et al*., 2001; Walsh *et al*., 2001), are used as forage legumes (Puckridge and French, 1983; Anon., 1988; Prosperi *et al*., 1989; Chatterton and Chatterton, 1996), have potential as summer annual forages (Zhu *et al*., 1996; Shrestha *et al*., 1998; de Haan *et al*., 2002), and/or can be used as intercrops with grains (de Haan *et al*., 1997), can be used as smother crops (de Haan *et al*., 1997; Sheaffer *et al*., 2002), are useful as over-winter cover crops (Fisk *et al*., 2001), or can be used as a 'disease break' for rotational crops (Walsh *et al*., 2001).

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Annual *Medicago* spp. originated in the Mediterranean region, in which the greatest species diversity occurs (Piano and Francis, 1992). Some 33 species of annual *Medicago* are recognized (Lesins and Lesins, 1979). Of these, *M. polymorpha* is considered to be ubiquitous; *M. truncatula, M. orbicularis* and *M. littoralis* are distributed throughout areas with Mediterranean-type climates; while some species have close associations with specific regions, such as *M. murex* with central and western Mediterranean regions, *M. rigidula* with the Irano-Turanian region, *M. tornata* and *M. aculeata* with the western Mediterranean, and *M. blancheana, M. constricta, M. noeana, M. radiata* and *M. rotata* with the eastern Mediterranean region (Piano and Francis, 1992).

The distribution of annual *Medicago* spp. is influenced by edaphic factors including: soil type, levels of soil Ca, P, S and NO_3, and soil salinity (Andrew, 1977; Robson, 1983; Abdelguerfi *et al*., 1988; Prosperi *et al*., 1989; Ehrman and Cocks, 1990; Ewing and Robson, 1990; Piano *et al*., 1991; Bounjemate *et al*., 1992); climatic factors such as altitude and annual rainfall (Gintzburger and Blesing, 1979; Francis, 1980, 1987; Cocks and Ehrman, 1987; Abdelguerfi *et al*., 1988; Ehrman and Cocks, 1990; Piano *et al*., 1991); biotic factors such as regeneration capacity and levels of hardseededness (Francis, 1987; Thomson *et al*., 1990); and possibly also by available

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rhizobial components (Robson and Loneragan, 1970; Robson, 1983; Vincent, 1988). The effects of these factors on the distribution of annual *Medicago* spp. have previously been reviewed, for example by Piano *et al.* (1991) and by Piano and Francis (1992).

There are numerous pathogens of forage plants, with some 400 fungal, bacterial, viral, mycoplasma and nematode diseases known to affect forage species on a worldwide basis (Haggar *et al*., 1984). Although some aspects of forage diseases have been extensively reviewed (e.g. Graham *et al*., 1979; Haggar *et al*., 1984; Barnett and Diachun, 1985; Braverman *et al*., 1986; Edwardson and Christie, 1986; Johnstone and Barbetti, 1987; Raynal *et al*., 1989; Cook and Yeates, 1993; Lenné, 1994*a, b*; Barbetti *et al*., 1996), such reviews cover superficially, if at all, necrotrophic fungal diseases of annual *Medicago* spp.

This review outlines the major diseases caused by foliar and soil-borne necrotrophic pathogens and explores the potential for using the spectrum of necrotrophic pathogen–host interactions, with annual *Medicago* as the host plant, to better understand and model pathosystems within the diseases caused by nectrotrophic pathogens across forage and grain legumes. The links between annual and perennial *Medicago* spp., the model plant *M. truncatula*, and with grain legume species, in relation to their interactions with one or more related or unrelated necrotrophic pathogens are considered. For this review, we based our definition of 'necrotrophs' on that of Agrios (2004), to mean organisms which can have one part of their life cycle on dead host/tissue and which can grow on artificial nutrient media. As much of the existing literature covers one or more annual *Medicago* spp. in addition to *M. truncatula*, discussions in this review relate to annual *Medicago* spp. in general.

NECROTROPHIC PATHOGENS

There are many foliar and soil-borne necrotrophic pathogens of annual *Medicago* spp., and an outline of some of the most important and/or frequently occurring diseases in different regions that are caused by necrotrophic fungal pathogens are listed in Table 1. As indicated, some necrotrophic fungal pathogens (e.g. *Phoma medicaginis*) on annual *Medicago* spp. are also known to stimulate production of high levels of phyto-oestrogenic compounds, such as coumestrol. These compounds can adversely affect ovulation rates in sheep (Smith *et al*., 1979; Croker *et al*., 1994*a, b*, 1999, 2005). It is noteworthy that production of phyto-oestrogen compounds in response to disease varies between genotypes of annual *Medicago* spp. (Barbetti and Fang, 1991; Barbetti and Nichols, 1991).

As also indicated in Table 1, a number of *Fusarium* spp. on annual *Medicago* spp. have been shown to be responsible for the production of deleterious mycotoxins on animal feed material in Australia (Barbetti and Allen, 2005, 2006) and particularly in South Africa, where there is concern about the ability of *Fusarium* spp. on annual *Medicago* spp. to produce mycotoxins on animal feed material (Lamprecht *et al*., 1986).

HOST RESISTANCE TO NECROTROPHIC PATHOGENS

A range of cultural and chemical strategies have been used to varying degrees for control of diseases in annual *Medicago* spp. (e.g. Barbetti, 1989*a*; You *et al*., 1999; Walsh *et al*., 2001). However, it is the use of resistant cultivars that offers the most effective long-term control measure for annual forage and grain legume diseases. This approach has been spectacularly successful as the main and most reliable avenue for the successful management of the most important pathogens of annual forage legumes such as *Kabatiella* on *Trifolium* spp. (Barbetti, 1996). These same opportunities exist for at least some diseases, such as Phoma blackstem disease, in annual *Medicago* spp. (Barbetti, 1993) providing large-scale screening of germplasm for resistance is undertaken as has been done for many years against *Kabatiella* in Australia (Nichols *et al*., 1996).

A degree of resistance to many diseases is already available in some annual *Medicago* spp. For example, variation in resistance to stem and leaf disease caused by *Phoma medicaginis* (Renfro and Sprague, 1959; Barbetti, 1987, 1989*a*, 1990; O'Neill *et al*., 2003), by *Leptosphaerulina trifolii* (Renfro and Sprague, 1959; Martinez and Hanson, 1963; Barbetti and Nichols, 1991), by *Colletotrichum trifolii* (Parmelee, 1962; Raynal, 1977; Elgin and Ostazeski, 1982; Lamprecht and Knox-Davies, 1984*a, b*; Lamprecht, 1986*b*; Troeung and Gosset, 1990; O'Neill and Bauchan, 2000), by *Cercospora medicaginis* (Berger and Hanson, 1963; Barbetti, 1985), by *Pseudopeziza medicaginis* (Schmiedeknecht, 1959), by *Phytophthora medicaginis* (de Haan *et al*., 2002) and by *Leptotrochila medicaginis* (Semeniuk and Rumbaugh, 1976) has been reported in annual *Medicago* spp. Andrew (1962) demonstrated that *M. denticulata* had much greater resistance to post-emergence by *Pythium* spp. than did *M. minima*.

Methods of identifying host resistance: foliar necrotrophic pathogens

Various types of tests have been used to identify host resistance in annual *Medicago* spp. to necrotrophic foliar pathogens, ranging from laboratory to glasshouse screening tests to evaluations of swards under field conditions as illustrated in Fig. 7. For example, Troeung and Gosset (1990) used laboratory tests to locate resistance to *Colletotrichum trifolii* in annual *Medicago* spp. such as *M. rigidula* and *M. truncatula*. Lamprecht and Knox-Davies (1984*b*) tested the reactions of 3- and 6-week-old plants of annual *Medicago* accessions using *C. trifolii* spore suspensions at 15–25 °C under glasshouse conditions and assessed disease using the five-point scale of Ostazeski *et al*. (1969) (immume, resistant, intermediate, susceptible, dead) after 14 d to highlight different accession responses to crown rot. Lamprecht (1986*b*) used five different *C. trifolii* spore suspension application techniques and a sand-bran culture application technique on 4- to 6-week-old injured and uninjured *M. littoralis,*

TABLE 1. *Foliar and soil-borne disorders of annul* Medicago *spp., the causal pathogens, the regions of their occurrence and key references*

Disease	Pathogen	Region	Reference
Foliar			
Phoma black stem	*Phoma medicaginis*	Europe	Sampson and Western (1941)
		USA	Graham *et al*. (1979), O'Neill *et al.* (2003)
		South Africa	Lamprecht and Knox-Davies (1984*a*)
		Australia	Barbetti (1983), Johnstone and Barbetti (1987), Barbetti and Fang (1991), Barbetti and Nichols (1991), Barbetti (1992, 1993) (see Figs 1 and 2 for typical disease symptoms)
Anthracnose	*Colletotrichum trifolii*	South Africa	Lamprecht and Knox-Davies (1984*a, b*), Lamprecht (1986*b*)
		Canada	Parmelee (1962)
		USA	O'Neill and Bauchan (2000)
		Europe	Raynal (1977), Troeung and Gosset (1990)
		Australia	Mackie *et al*. (1999)
Leptosphaerulina leaf and stem spot	*Leptosphaerulina trifolii* (syn. *L. briosiana*)	South Africa	Lamprecht and Knox-Davies (1984*a*)
		USA	Renfro and Sprague (1959), Martinez and Hanson (1963), Graham *et al*. (1979)
		Australia	Barbetti (1989*b*)
Pseudopeziza leaf spot	*Pseudopeziza medicaginis*	South Africa	Lamprecht and Knox-Davies (1984*a*)
		Australia	Barbetti (1989*b*); Mackie *et al*. (1999) (see Fig. 3 for typical disease symptoms)
Stemphylium leaf spot	*Stemphylium botryosum* and *S. vesicarium*	USA	Chilton *et al*. (1943), Renfro and Sprague (1959)
		South Africa	Lamprecht *et al*. (1984)
		Australia	Barbetti (1989*b*), Mackie *et al*. (1999)
Stagonospora leaf spot	*Stagonospora meliloti*	Western Australia	Barbetti (1983, 1989*b*) (see Fig. 4 for typical disease symptoms)
Cercospora leaf spot	*Cercospora medicaginis*	USA	Berger and Hanson (1963)
		Australia	Barbetti (1985)
Yellow leaf blotch	*Leptotrochila medicaginis*	North America	Semeniuk and Rumbaugh (1976)
High levels of phyto-oestrogenic compounds, such as coumestrol	*Phoma medicaginis*	Australia	Francis and Millington (1971), Collins and Cox (1984), Barbetti and Fang (1991), Barbetti and Nichols (1991), Barbetti (1993, 1995*a*)
Soil-borne			
Decline	Various	South Africa	Lamprecht *et al*. (1988)
		USA	Thies and Barnes (1991), de Haan *et al.* (2002)
		Australia	Andrew (1962), Kollmorgen (1974), Kellock *et al*. (1978), Bretag (1985), Bretag and Kollmorgen (1986), Mebalds (1987), Barbetti (1989*c*), Neal *et al*. (1997) (see Figs 5 and 6 for typical root disease symptoms on seedlings and adult plants, respectively)
	Fusarium avenaceum	Australia	Kollmorgen (1974), Bretag (1985), Bretag and Kollmorgen (1986), Mebalds (1987), Barbetti (1989*c*)
	F. acuminatum, F. avenaceum	Australia	Kollmorgen (1974)
	Phoma medicaginis, Pythium irregulare, Rhizoctonia solani, Pythium spp.	Australia	Andrew (1962)
	Species of *Pythium, Fusarium* and *Phoma*	Australia	You *et al.* (2000)
Root rot	*Phytophthora clandestina*	Australia	Clarke and Greenhalgh (1986), Barbetti (1989*c*)
	Fusarium avenaceum, F. culmorum, F. graminearum and *F. lateritium*	South Africa	Lamprecht *et al*. (1988)
	Pythium irregulare, P. ultimum and *P. spinosum*	South Africa	Lamprecht *et al*. (1988)
	Cylindrocladium scoparium	South Africa	Lamprecht (1986*a*)
	Phytophthora medicaginis	USA	Thies and Barnes (1991), de Haan *et al.* (1996, 2002)
Mycotoxin	*Fusarium* spp.	Australia	Barbetti and Allen (2005), Barbetti and Allen (2006)
		South Africa	Lamprecht *et al.* (1986)

M. tornata, M. truncatula and *M. scutellata* plants held at 15–28 °C under glasshouse conditions and assessed disease on a 0–4 pointscale, where 4 indicated crown completely rotted, to successfully highlight resistance in *M. truncatula*. O'Neill and Bauchan (2000) and O'Neill *et al.* (2003) used standardized environmental conditions in growth chambers to screen separately 201 accessions across 36 annual *Medicago* spp. for resistance to *C. trifolii*

FIG. 1. Phoma blackstem on *Medicago truncatula* 'Jemalong'.

FIG. 2. Phoma blackstem on *Medicago polymorpha* 'Serena'.

FIG. 3. Pseudopeziza leaf spot on *Medicago polymorpha* 'Circle Valley'.

FIG. 4. Stagonospora leaf spot on *Medicago polymorpha* 'Serena'.

FIG. 5. Severe rotting of roots of *Medicago polymorpha* seedlings.

and *Phoma medicaginis*. Semeniuk and Rumbaugh (1976) screened 25 annual *Medicago* spp. for resistance to *Leptotrochila medicaginis* under glasshouse conditions, identifying a high level of resistance in 22 of the species tested.

Frequently, germplasm responses to various diseases are a consequence of opportunistic occurrences of diseases in germplasm trials established for other purposes. A good example of this was the assessment by Lamprecht and Knox-Davies (1984*a*) of annual *Medicago* spp. varietal reactions (using a 0–4 disease severity index where 4 represents maximum disease) to a range of necrotrophic pathogens (*Phoma medicaginis, Leptosphaerulina trifolii, Colletotrichum trifolii* and *C. destructivum, Cercospora medicaginis, Pseudopeziza medicaginis* and *Stemphylium*

FIG. 6. Root disease on established *Medicago polymorpha* plants.

FIG. 7. Field swards of species of annual *Medicago* for identification of varietal resistances or susceptibilities to Phoma blackstem disease. Note yellowing and poor growth of the most susceptible varieties.

vesicarium) in experimental field plots at 12 locations in South Africa. In Western Australia, a 0–10 point disease assessment scale is frequently used (Barbetti, 1990), and for leaf pathogens such as *Phoma, Leptosphaerulina, Pseudopeziza*, plots are rated 0, where there was no disease; 1, where there was >0–9 %; 2, ⩾10–19 %; 3, ⩾20–29 %; 4, ⩾30–39 %; 5, ⩾40–49 %; 6, ⩾50–59 %; 7, ⩾60–69 %; 8, ⩾70–79 %; 9, ⩾80–89 %; and 10, ⩾90 % of leaves with lesions. However, depending upon the disease symptoms, especially if multiple diseases are occurring in the same plots, diseases can also be rated using a simplified system where 0 = no disease, 1 = there were >0–10 % of stems with mostly small (<3 mm diameter or length) lesions; 2 = there were >10–20 % of stems with small lesions; 3 = there were >20–30 % of stems with lesions and then ratings 4–10 reflecting an increasing incidence and severity of damage and, ultimately, complete collapse of the sward.

Methods of identifying host resistance: soil-borne necrotrophic pathogens

Various types of tests have been used to identify host resistance in annual *Medicago* spp. to necrotrophic soil-borne pathogens, ranging from glasshouse screening tests to evaluations under field conditions. For example, de Haan *et al*. (2002) used field assays on single rows of annual *Medicago* spp. at a site that had been artificially prior-inoculated using infested soil. They applied irrigation to maximize disease severity and assessed the level of root rot on plants using the 1–6 point disease scoring system described by Thies and Barnes (1991), which defined resistant germplasm as those with disease scores of only 1–2.

It is important that resistance to seedling death, damping-off and root disease all be determined in the search for annual *Medicago* spp. genotypes with improved field performance against soil-borne pathogens in disease-prone areas. This is because studies with annual *Trifolium* spp. have clearly indicated that resistance to seedling damping-off is frequently specific and under different genetic control to resistance to root rot (e.g. You *et al*., 2005), and it is likely that the same will apply for annual *Medicago* spp.

Sources of host resistance

Identification of host resistance to many necrotrophic pathogens has frequently been a key aim of cultivar

development programmes, especially as there is large variability in annual *Medicago* spp. in relation to disease resistance, for example Phoma blackstem disease (Barbetti, 1989*a*, 1990, 1993; O'Neill *et al*., 2003). However, such resistance has rarely been defined (e.g. as complete or partial, under a monogenic or polygenic control, respectively).

In the United States, de Haan *et al*. (2002) identified three accessions of *M. polymorpha* that had resistance to *Phytophthora medicaginis*. O'Neill *et al.* (2003) identified accessions within *M. constricta, M. doliata, M. heyniana, M. laciniata, M. lesinsii, M. murex, M. orbicularis, M. praecox, M. soleirolii* and *M. tenoreana* that exhibited a high level of resistance to *Phytophthora medicaginis*. O'Neill and Bauchan (2000) identified 14 accessions of the 201 tested that had resistance to *Colletotrichum trifolii*, including accessions in *M. murex, M. muricoliptis, M. polymorpha* var. *brevispina, M. polymorpha* var. *polymorpha, M. radiata, M. soleirolii, M. truncatula* and *M. turbinata.*

In South Africa, Lamprecht (1986*b*) identified *M. truncatula* cultivars 'Borung' and 'Cyfield' as the most resistant of nine cultivars tested from across four annual *Medicago* spp. (*M. littoralis, M. tornata, M. truncatula* and *M. scutellata*).

In Australia, a high degree of resistance, particularly to Phoma black stem, has been identified in screening programmes, useful both as parental materials in breeding and in providing resistant cultivars (Barbetti, 1995*b*). The incorporation of this resistance in commercial cultivars offers a promising long-term control measure for foliar disease in annual *Medicago* spp. (Barbetti and Nicholas, 1997).

Variation in resistance to stem and leaf disease caused by *Pseudopeziza medicaginis* (Renfro and Sprague, 1959; Barbetti, 1987, 1989*a*, 1990) and *L. trifolii* (Renfro and Sprague, 1959; Barbetti and Nichols, 1991) has been reported in annual *Medicago* spp.

It is noteworthy that the Mediterranean region has proved to be a productive source for collecting host germplasm with excellent resistance to one or more foliar and soil-borne necrotrophic pathogens. The value of this region as a source of resistance is highlighted by the example of the four introductions of *Trifolium subterraneum* germplasm from Sardinia that were directly released as new cultivars in Australia in the early 1990s, namely cultivars 'Denmark', 'Goulburn', 'Leura' and 'York'. Against important diseases on *T. subterraneum* in Australia, two of these cultivars had good resistance to both Race 1 and Race 2 of *K. caulivora*, two had good resistance to *Uromyces trifolii-repentis*, three had resistance to *Cercospora zebrina* and all four had outstanding resistance to the original race of *Phytophthora clandestina*. This is despite the fact that these diseases occurred infrequently (e.g. *C. zebrina* and *U. trifolii-repentis*) or have never occurred (e.g. *K. caulivora* and *P. clandestina*) in Sardinia, highlighting the value of looking for annual *Medicago* spp. sources of resistance to necrotrophic pathogens from the Mediterranean centre of origin, even if the particular diseases of interest do not occur there (Barbetti, 1996; Nichols *et al*., 1996; M. J. Barbetti, unpubl. data). It is interesting that nearly all the major diseases of annual *Medicago* spp., especially in Australia, are caused by necrotrophic pathogens that are common to other pasture legumes and across several continents, including the centre of origin of these host taxa. Presumably, such wide distribution of many of these pathogens is due to their wide host range and/or to their seed-borne nature. However, some annual *Medicago* and *Trifolium* species in Australia can be challenged by a pathogen such as *Phytophthora clandestina* that is only recorded in Australia and is yet to be recorded in the centre of origin of the host taxa. This can be described as a 'new encounter disease' (Buddenhagen, 1977; Allen *et al*., 1998) for both these legumes. With *Phytophthora clandestina*, there is clear indication of the existence of allopatric resistance (Allen *et al*., 1998) in Sardinia. It is also interesting that secondary centres of genetic diversity for annual *Medicago* and *Trifolium* spp. in Australia have provided successful sources of naturalized strains with increased resistance to disease for pasture legumes (Barbetti, 1996).

MEDICAGO TRUNCATULA: THE FIRST MODEL *MEDICAGO* SP.

M. truncatula*: why it is an ideal model for necrotrophic pathogens*

Although *Medicago* spp. are subject to a very large range of foliar as well as soil-borne necrotrophic pathogens, our knowledge of resistance sources, resistance expression, genetic determination and resistance mechanisms is still inadequate. However, annual *Medicago* spp. are now emerging as appropriate and agronomically relevant plants to study necrotrophic pathogens. Among the annual *Medicago* spp., *M. truncatula* is already proving to be an ideal model plant for both functional and structural genomic studies for identifying agronomically important genes and studying pathogen relationships in legumes. *Arabidopsis*, which to date has been the most valuable model plant to work on genetics and physiology of various aspects of plant biology, is a host of a very restricted range of species of necrotrophic pathogens, and is a less relevant model plant as a result.

Medicago truncatula is being used as a model plant for use in both molecular and classical genetic studies because of its ideal characteristics, such as its small, diploid genome, rapid generation time, self-fertility and ease of seed production (Cook, 1999). *M. truncatula* has proven to be an easily transformed species, ensuring its role as an ideal model system for investigating and elucidating gene function in legume species (Trieu *et al*., 2000). There are now several large-scale international projects that have been initiated in relation to *M. truncatula* genomics, including the complete sequence description through an international consortium. Databases considering whole genome sequencing and annotation, expressed sequence tags (ESTs), structural genomics and comparative mapping, bacterial artificial chromosome

(BAC) libraries and physical maps, gene expression, metabolic profiling and bioinformatic tools are accessible through the http://www.medicago.org/ website, which offers adequate links to other sites dealing with *M. truncatula* genomic resources, and legume phylogeny, protocols and publications. There is no resource specifically dedicated to disease resistance in *M. truncatula* as yet, apart from some reported EST libraries constructed while submitting plants to various biotic stresses (Torregrossa *et al*., 2006).

The close phylogenetic relationship of *M. truncatula* to perennial cultivated *Medicago* spp. such as alfalfa, and other legumes such as pea (*Pisum sativum*), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*) and faba bean (*Vicia faba*) increases the attractiveness of utilizing *M. truncatula* to improve our understanding of important agronomic traits in related grain and forage legume species (Dénarié, 2002). Large crop losses in these grain legumes are due to various necrotrophic pathogens (Tivoli *et al*., 2006). Use of *M. truncatula* as a model system offers the first real opportunity for understanding the complex genetic and physiological basis of host–pathogen interactions, including secondary metabolite signalling, as has been done earlier for *Arabidopsis thaliana* (Bouwmeester *et al*., 2003). It can also be used for transferring these insights to other annual *Medicago* spp., to grain legumes and to perennial cultivated *Medicago* spp. In addition, being a field crop species (unlike *Arabidopsis*) it could be readily used directly in the field where appropriate.

M. truncatula*: a host of most necrotrophic pathogens affecting* Medicago *spp.*

Large-scale screening was recently undertaken of *M. truncatula*, using the significant natural variation in the Australian *Medicago* spp. collection, to assess differential responses to over 25 necrotrophic pathogens including those causing foliar diseases such as Ascochyta blight, Botrytis grey mould, Colletotrichum anthracnose and Phoma black stem, and root diseases including those caused by *Fusarium* and *Rhizoctonia* (Ellwood *et al*., 2001, 2004, 2005*a, b*). This clearly shows that *M. truncatula* is suitable as a host for a very large range of necrotrophic pathogens and that variation for resistance can be identified for some of these pathogens within the Australian *Medicago* spp. germplasm resources. The United States Department of Agriculture houses a *Medicago* spp. core collection. Within this, of 201 accessions of annual *Medicago* species, studied by O'Neill and Bauchan (2000) and O'Neill *et al.* (2003), most were observed to be susceptible to Phoma black stem and leaf spot caused by *P. medicaginis* and also to anthracnose caused by *C. trifolii*. No source with a high level of resistance could be found against *P. medicaginis*, but accessions with a high level of resistance to *C. trifolii* were identified (O'Neill and Bauchan, 2000). The screening of four *M. truncatula* lines by Torregrossa *et al*. (2004) identified cultivar 'Jemalong' and line DZA315.16 as resistant to *C. trifolii* Race 1. Therefore, *M. truncatula* can be used as a model for most necrotrophic pathogens affecting annual and perennial *Medicago* spp. Disease screening studies involving diseases currently associated with losses in other grain and forage legume species and/or genera have also been undertaken. For example, screenings within *Medicago* spp. have provided key leads in relation to resistance to the fungus *Mycosphaerella pinodes*, which is responsible for ascochyta blight (black spot) on pea (Moussart *et al*., 2006). From a core collection of 131 natural populations (Prosperi *et al*., 2002), 34 *M. truncatula* ecotypes and nine ecotypes belonging to other *Medicago* spp. were screened for *My. pinodes* resistance either on plantlets or on detached leaves. High levels of resistance to *My. pinodes* with reduced variability between *M. truncatula* accessions have now been identified (Tivoli *et al*., 2005; Moussart *et al*., 2006), where although some flecks were observed on leaves on all the screened accessions, most accessions showed significant restriction of lesion development. Slowly progressing lesions with fructifications were observed on only a few accessions. Three different resistance reactions were noted, including absence of lesions, restricted fungal development to the site of inoculum application and cessation of progressive lesion development (Moussart *et al*., 2006). These resistance reactions have also been confirmed in another set of *M. truncatula* accessions (D. Rubiales, pers. comm.).

The availability of germplasm showing various resistance levels towards necrotrophic pathogens within the *M. truncatula* collections opens the way to (1) isolate the genes controlling resistance to legume necrotrophic pathogens, and (2) study host–pathogen interactions at the histological, biochemical and physiological levels.

M. truncatula*: provides for the isolation of genes controlling resistance in* Medicago *and grain legume species*

A cross between cultivar 'Jemalong' and the line F83005.5 allowed genetic analysis of resistance in an F_2 population, indicating that resistance to *C. trifolii* was dominant and controlled either by a major resistance gene showing a distorted segregation or by more than one gene (Torregrossa *et al*., 2004). Ellwood *et al*. (2005*b*) have developed and genetically mapped 80 polymorphic markers in a different F_2 population from a cross segregating for resistance to *P. medicaginis* and showed that resistance is under the control of a major gene. Therefore, a rather simple genetic control has been shown in *M. truncatula* for resistance to two major necrotrophic pathogens of *Medicago* spp., namely *C. trifolii* and *P. medicaginis*. This opens up the way not only for gene isolation based on genomic resources developed in *M. truncatula* but also for comparative analysis of resistance expression, genetic control and resistance mechanisms between *M. truncatula* and other *Medicago* spp. and grain legumes.

The model plant *M. truncatula* can constitute an efficient bridge between grain and forage legumes. Synteny between *Medicago* spp. as well as between *Medicago* and grain legumes such as pea (Gualtieri *et al*.,

2002; Kalo *et al*., 2004) should allow a comparative analysis of genes or quantitative trait loci involved in resistance to necrotrophic pathogens in these species, including the number of genes involved, genomic localization, contribution to variation, etc. This same perspective could easily be applied where pathogens are common to certain other species (such as *C. trifolii* and *P. medicaginis* between different *Medicago* spp., and probably also *P. medicaginis* var. *pinodella* and *My. pinodes* and between *M. truncatula* and *P. sativum*). The suitability of such an approach is illustrated by a study by Kamphuis *et al.* (2005) in which they were able to conduct disease screening and genetic analyses in *M. truncatula* for resistance to different *Phoma* species taken from different legume crops.

M. truncatula*: boosts understanding of host–pathogen interactions in both* Medicago *spp. and grain legumes*

During pathogenic interactions between annual *Medicago* spp. and *C. trifolii*, O'Neill and Bauchan (2000) observed resistant reactions that were similar to those found in incompatible interactions between this pathogen and alfalfa, with fungal development severely restricted to host tissues at the point of inoculation. They suggested a hypersensitive response, possibly involving phytoalexins, as in the *M. sativa–C. trifolii* pathosysten (O'Neill, 1996). Cytological observations by Torregrossa *et al*. (2004, 2006) also showed that resistance was linked to a hypersensitive reaction. Subsequent microarray analysis showed that a strong correlation existed between the number of up-regulated genes and the resistance phenotype. Large differences appeared at 48 h post-inoculation and a large number of defence genes were involved.

Toyoda *et al*. (2004) described the *M. truncatula–My. pinodes* interaction and suggested it as a new pathosystem for genetic dissection of susceptibility to fungal pathogens. They observed that as on peas, attenuation or suppression of host defences contributes to establishment of susceptibility. In the case of ascochyta blight resistance on chickpea, Muehlbauer *et al.* (2005) compared the sequences from selected chickpea BAC clones with *M. truncatula* genome sequences. They successfully identified several orthologous contigs. The same approach, based on comparative genomics and synteny analyses, was developed by Ford *et al*. (2005) for ascochyta blight resistance on lentils.

An instructive view of the relevance of *M. truncatula* as a model species for *Medicago* spp. as well as for grain legume pathogens is presented by the example of *Aphanomyces euteiches*, a pathogen causing severe root rot and important yield losses on grain legumes (pea, lentil, vetch, etc.) as well as on alfalfa. The screening of the French core collection (Prosperi *et al*., 2002) showed that there is a large variation within *M. truncatula* ecotypes for resistance to a pea *A. euteiches* isolate, and that the *M. truncatula* resistant ecotypes present a much higher level of resistance than that observed on pea (Pilet-Nayel *et al*., 2005; Tivoli *et al*., 2005; Moussart *et al*., 2006). Such variation was previously reported in the US by Vandemark and Grunwald (2004) for *M. truncatula* accessions evaluated for resistance to *A. euteiches* Race 2, which is pathogenic to alfalfa. Genetic studies (Jacquet *et al*., 2005*a, b*; Pilet-Nayel *et al*., 2005), as well as cytological and transcriptomic studies (Jacquet *et al*., 2005*a, b*), have been developed using susceptible and resistant lines of *M. truncatula*. In order to gain insight into molecular and physiological changes in diseased legume roots, a transcriptomic approach of *M. truncatula* during infection by *A. euteiches* was developed by Nyamsuren *et al*. (2003), which demonstrated that classical mechanisms of pathogenesis as well as new specific gene regulations are involved in root rot disease development caused by *A. euteiches*. Recently, Colditz *et al*. (2005) conducted a comparative proteomic analysis of the interaction formed between three lines of *M. truncatula* and a single *A. euteiches* strain. Several proteins were identified to be differentially induced after infection of the susceptible or resistant lines. This example shows that through complementary genetic and genomic-based strategies, such a pathosystem can be dissected within *M. truncatula* and comparative analysis subsequently carried out between this model species and other *Medicago* spp. and grain legumes to provide a better understanding of the nature of both resistance and susceptibility to necrotrophic fungal pathogens.

CONCLUSIONS

Host resistance clearly offers the best strategy for cost-effective, long-term control of nectrotrophic foliar and soil-borne pathogens, particularly as useful resistance to a number of these diseases has been identified in annual *Medicago* germplasm collections. Previously, *Arabidopsis* had been the most valuable model plant to work on genetics and physiology of various aspects of plant biology, including host–pathogen relationships. However, recently and initially, the annual plant *Medicago truncatula* has emerged as a more appropriate and agronomically relevant substitute as a model plant for legumes. Because it is both a host of foliar and soil-borne fungal necrotrophic pathogens of numerous *Medicago* spp. in addition to several necrotrophic fungal pathogens of other grain legumes, *M. truncatula* is proving an excellent model upon which to dissect and to understand the mechanisms of resistance to necrotrophic pathogens of legumes in general. For example, the genetic and genomic resources now available in relation to *M. truncatula* will boost genetic analyses in grain legume species and therefore contribute to improved understanding of the structure and function of host resistance genes in relation to necrotrophic fungal pathogens across forage and grain legumes.

Important reasons for annual *Medicago* spp. proving to be ideal model crops include the following. First, these species are at the 'meeting point' between the model plant *M. truncatula*, other annual *Medicago* spp., perennial *M. sativa* and various grain legumes species, with several

necrotrophic fungal pathogens able to attack more than one of annual or perennial *Medicago* and grain legume species (e.g. *A. euteiches, C. trifolii* and *P. medicaginis*). Secondly, genetic studies undertaken in relation to identification of resistance genes to fungal necrotrophic pathogens can be easily transferred to *M. truncatula* not only from other annual or perennial *Medicago* spp., but also from grain legumes. Parallel approaches can now be undertaken across both annual and perennial *Medicago* species and grain legumes to provide better understanding of the nature of resistance, particularly for related necrotrophic fungal pathogens such as *P. medicaginis* and *My. pinodes*, the former a pathogen on annual and perennial *Medicago* spp. and pea, the latter a pathogen only on pea. In future, closer consideration of the common links between *M. truncatula*, other annual and perennial *Medicago* spp., and other grain legumes will not only enable wider application of genetic and genomic approaches to be undertaken on legumes, but also hasten transfer of relevant knowledge on necrotrophic pathogens between *Medicago* as the model genus, *M. truncatula* as the model plant and grain legumes. For example, improved understanding of key plant–pathogen interactions has already been demonstrated in relation to resistance against *A. euteiches* and *C. trifolii*. Clearly, modelling of host–pathogen interactions in annual *Medicago* spp., including *M. truncatula*, will greatly improve our understanding of the nature of both resistance and susceptibility to necrotrophic fungal pathogens across forage and grain legumes. This will undoubtedly help in the development of new forms of resistance in legumes to a variety of necrotrophic pathogens.

LITERATURE CITED

Abdelguerfi A, Chapot JY, Conesa AP. 1988. Contribuution a l'etude de la répartition des luzernes annuelles spontanées en Algerie selon certains facteurs du milieu. *Fourragères* **113**: 89–106.

Agrios GN. 2004. *Plant pathology*, 5th edn. New York: Academic Press, Harcourt Brace Jonanovich, Publishers.

Allen DJ, Lenne JM, Wood, D. 1998. New encounter diseases and allopatric resistance. In: *Proceedings of the 7th International Congress of Plant Pathology*, paper 4.1.5.

Andrew CS. 1977. The effect of sulphur on the growth, sulphur and nitrogen concentrations, and critical sulphur concentrations of some tropical and temperate pasture legumes. *Australian Journal of Agricultural Research* **28**: 807–820.

Andrew WD. 1962. Susceptibility to damping-off in seedlings of *Medicago denticulata* Willd. and *M. minima* (L.) Bart. during the early post-emergence period. *Australian Journal of Biological Sciences* **16**: 281–283.

Anon. 1988. *Pasture, Forage and Livestock Program.* Annual Report 1988. Aleppo, Syria: ICARDA.

Barbetti MJ. 1983. Fungal foliage diseases of pasture legumes. *Journal of Agriculture, Western Australia* **1**: 10–12.

Barbetti MJ. 1985. Infection studies with *Cercospora zebrina* on pasture legumes in Western Australia. *Australian Journal of Experimental Agriculture* **25**: 850–855.

Barbetti MJ. 1987. Effects of temperature and humidity on disease caused by *Phoma medicaginis*, resistance in some *Medicago* cultivars and the incidence of seed-borne inoculum. *Australian Journal of Experimental Agriculture* **27**: 851–856.

Barbetti MJ. 1989*a*. Strategies for control of Phoma black stem in annual *Medicago* species. *Australian Journal of Experimental Agriculture* **29**: 635–640.

Barbetti MJ. 1989*b*. *Fungal diseases of pasture legumes in Western Australia.* Bulletin No. 4133. Perth: Department of Agriculture, Western Australia.

Barbetti MJ. 1989*c*. Response of *Medicago* cultivars to fungal root pathogens associated with *Trifolium subterraneum. Plant Protection Quarterly* **4**: 1–3.

Barbetti MJ. 1990. Resistance in annual *Medicago* species to *Phoma medicaginis* under controlled environment and field conditions. *Australian Journal of Experimental Agriculture* **30**: 209–214.

Barbetti MJ. 1992. Efficacy of fungicides for control of Phoma black stem and Pseudopeziza leaf spot in annual *Medicago polymorpha* var. *brevispina*. *Plant Protection Quarterly* **7**: 21–22.

Barbetti MJ. 1993. Phoma black stem disease—a threat to susceptible annual medic pastures. In: *Proceedings of the XVII International Grasslands Congress*, 929–930.

Barbetti MJ. 1995*a*. Relative resistance, associated yield losses and phyto-oestrogen production from fungal foliar diseases in new and old annual *Medicago* cultivars. *Australian Journal of Agricultural Researc*h **46**: 441–450.

Barbetti MJ. 1995*b*. Resistance in annual *Medicago* species to *Phoma medicaginis* and *Leptospherulina trifolii* under field conditions. *Australian Journal of Experimental Agriculture* **35**: 209–214.

Barbetti MJ. 1996. The successful control of clover scorch disease (*Kabatiella caulivora*) in Australian subterranean clover pastures. In: Chakraborty S, Leath KTL, Skipp RA, *et al.*, eds. *Pasture and forage crop pathology*. Madison, WI: ASA, CSSA and SSSA, 589–601.

Barbetti MJ, Allen JG. 2005. Association of *Fusarium* species, with potential for mycotoxicosis, on pods of annual *Medicago* in Western Australia. *Australian Journal of Agricultural Research* **56**: 279–284.

Barbetti MJ, Allen JG. 2006. Association between toxigenic *Fusarium* species on annual medic pastures and reduced feed intake by sheep in Western Australia. In: *Proceedings of the 7th International Symposium on Poisonous Plants* (in press).

Barbetti MJ, Fang CS. 1991. Relationship between Phoma black stem severity and seed yield and coumestrol content in three *Medicago polymorpha* var. *brevispina* cultivars. *Australian Journal of Agricultural Research* **42**: 409–415.

Barbetti MJ, Nicholas DA. 1997. Effect of Phoma black stem and pepper spot diseases on yield, regeneration, sward composition and phytooestrogen levels in grazed annual medic pastures. In: *Proceedings of the Australasian Plant Pathology Society Biennial Conference,* 65.

Barbetti MJ, Nichols PGH. 1991. Effect of *Phoma medicaginis* and *Leptosphaerulina trifolii* on herbage and seed yield and coumestrol content of annual *Medicago* species. *Phytophylactica* **23**: 223–227.

Barbetti MJ, Jones RAC, Riley IT. 1996. Problems and progress in assessing direct and indirect yield losses caused by pathogens in pasture species. In: Chakraborty S, Leath KTL, Skipp RA, *et al.*, eds. *Pasture and forage crop pathology*. Madison, WI: ASA, CSSA and SSSA, 63–91.

Barnett OW, Diachun S. 1985. Virus diseases of clovers. In: Taylor NL, ed. *Clover science and technology*. Madison, WI: American Society of Agronomy, 235–268.

Berger RD, Hanson EW. 1963. Pathogenicity, host-parasite relationships, and morphology of some forage legume Cercosporae and factors related to disease development. *Phytopathology* **53**: 500–508.

Bounjemate M, Robson AD, Beale PE. 1992. Annual *Medicago* species in Morocco. II Distribution in relation to soil and climate. *Australian Journal of Agricultural Research* **43**: 751–763.

Bouwmeester HJ, Matusova R, Zhongkui S, Beale MH. 2003. Secondary metabolite signalling in host parasitic plant interactions. *Current Opinion in Plant Biology* **6**: 358–364.

Braverman SW, Lukezic FL, Zeiders KE, Wilson JB. 1986. *Diseases of forage grasses in humid temperate zones*. Bulletin No. 859. Pennsylvania: USDA, Agricultural Research Service.

Bretag TW. 1985. Fungi associated with root rots of annual *Medicago* spp. in Australia. *Transactions of the British Mycological Society* **84**: 329–334.

Bretag TW, Kollmorgen JF. 1986. Effects 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. *Australian Journal of Experimental Agriculture* **26**: 65–70.

Buddenhagen IW. 1977. Resistance and vulnerability of tropical crops in relation to their evolution and breeding. *Annals of the New York Academy of Sciences* **287**: 309–326.

Chatterton L, Chatterton B. 1996. *Sustainable dryland farming*. Cambridge: Cambridge University Press.

Chilton SJP, Henson L, Johnson HW. 1943. Fungi reported on species of *Medicago, Melilotus* and *Trifolium. United States Department of Agriculture, Miscellaneous Publication* 499.

Clarke RG, Greenhalgh FC. 1986. Screening for alternative hosts of *Phytophthora clandestina*. *Australasian Plant Pathology* **15**: 61–63.

Cocks PS, Ehrman TAM. 1987. The geographic origin of frost tolerance in Syrian pasture legumes. *Journal of Applied Ecology* **24**: 673–683.

Colditz F, Braun HP, Jacquet C, Niehaus K, Krajinski F. 2005. Proteomic profiling unravels insights into the molecular background underlying increased *Aphanomyces euteiches* tolerance of *Medicago truncatula*. *Plant Molecular Biology* **59**: 387–406.

Collins WJ, Cox RI. 1984. Oestrogenic activity in forage legumes. In: Barnes RF, Ball PR, Brougham RW, Marten GC, Minson DJ, eds. *Forage legumes for energy-efficient animal production. Proceedings of a trilateral workshop held in Palmerston North, New Zealand, April 30-May 4, 1984*. United States Department of Agriculture, Agricultural Research Service, 268–276.

Cook DR. 1999. *Medicago truncatula*: a model in the making. *Current Opinion in Plant Biology* **2**: 301–304.

Cook R, Yeates GW. 1993. Nematode pests of grassland and forage crops. In: Evans K, Trudgill DL, Webster JM, eds. *Plant parasitic nematodes in temperate agriculture*. Wallingford, UK: CAB International, 305–350.

Croker K, Barbetti MJ, Nichols PGH. 1994*a*. Incidence of coumestrol in medic pastures in Western Australia. *Proceedings of the Australian Society of Animal Production* **20**: 416

Croker K, Nichols PGH, Barbetti MJ, Adams N. 1994*b*. *Sheep infertility from pasture legumes*. Department of Agriculture Western Australia, Farmnote No. 6/94.

Croker K, Nichols PGH, Barbetti MJ, Adams N. 1999. *Sheep infertility from pasture legumes.* Department of Agriculture Western Australia, Farmnote No. 79/99.

Croker K, Nichols P, Barbetti MJ, Adams N. 2005. *Sheep infertility from pasture legumes.* Department of Agriculture Western Australia, Farmnote No. 41/2005.

Dénarié J. 2002. Genomics and genetics of *Medicago truncatula*: resources for legume crop improvement. *Grain Legumes* **38**: 20–21.

Edwardson JR, Christie RG. 1986. *Viruses infecting forage legumes*, Volumes I–III. University of California.

Ehrman TAM, Cocks PS. 1990. Ecogeography of annual legumes in Syria: distribution patterns. *Journal of Applied Ecology* **27**: 578–591.

Elgin JH, Ostazeski SA. 1982. Evaluation of selected alfalfa cultivars and related *Medicago* species for resistance to race 1 and race 2 anthracnose. *Crop Science* **22**: 39–42.

Ellwood SR, Parker K, Oliver RP. 2001. Disease resistance diversity to fungal necrotrophs in *Medicago truncatula*. In: *4th workshop on Medicago truncatula*.

Ellwood S, Kamphuis L, D'Souza N, Nair RM, Oliver RP. 2004. Resistance to fungal necrotrophs in *Medicago truncatula*. In: *Proceedings of the 5th European Grain Legumes Conference*, 343.

Ellwood S, Lichtenzveig J, Pfaff T, Kamphuis L, D'Souza N, Williams A, Oliver RP. 2005*a*. Resistance to fungal necrotrophs in *Medicago truncatula*. In: *Proceedings of the 2nd International Medicago workshop*, 24.

Ellwood S, Lichtenzveig J, Pfaff T. Kamphuis L, D'Souza N, Williams A. Oliver RP. 2005*b*. Resistance to fungal necrotrophs in *Medicago truncatula*. In: *Model Legume Congress 2005*, 100.

Ewing MA, Robson AD. 1990. The effect of nitrogen supply on the early growth and nodulation of several annual *Medicago* species. *Australian Journal of Agricultural Research* **41**: 489–497.

Fisk JW, Hesterman OB, Shrestha A, Kells JJ, Harwood RR, Squire JM, Sheaffer CC. 2001. Weed suppression by annual legume cover crops in no-tillage corn. *Agronomy Journal* **3**: 319–325.

Ford R, Taylor PWJ, Mustafa B, *et al*. 2005. Biotechnology applications for breeding lentil resistant to biotic and abiotic stresses. In: *Proceedings of the 2nd International Medicago workshop*, 22.

Francis CM. 1980. *Ecology and distribution of Medicago and some other legume species in the upper Jezira region of Iraq and Syria.* Western Australian Overseas Projects Authority, Consultant Report No. 2. Perth: Western Australian Department of Agriculture.

Francis CM. 1987. *Morocco, a plant collection tour.* Miscellaneous Report. Perth: Western Australian Department of Agriculture.

Francis CM, Millington AJ. 1971. The presence of methylated coumestans in annual *Medicago* species: response to a fungal pathogen. *Australian Journal of Agricultural Research* **22:** 75–80.

Gintzburger G, Blesing L. 1979. *Genetic conservation in Libya: indigenous forage legumes collection in northern Libya. Distribution and ecology of Medicago spp.* Part III. Rome: FAO-ARC TFUB, FAO.

Graham JH, Frosheiser FI, Stuteville DL, Erwin DC. 1979. *A compendium of alfalfa diseases.* St. Paul, MN: American Phytopathological Society.

Gualtieri G, Kulikova O, Limpens E, Kim D, Cook D, Bisseling T, Geurts R. 2002. Microsynteny between pea and *Medicago truncatula* in the SYM2 region. *Plant Molecular Biology* **50**: 225–235.

de Haan RL, Sheaffer CC, Samac DA. 1996. First report of *Phytophthora medicaginis* causing Phytophthora root rot on annual *Medicago* spp. *Plant Disease* **80**: 710.

de Haan RL, Sheaffer CC, Barnes DK. 1997. Effect of annual medic smother plants on weed control and yield in corn. *Agronomy Journal* **89**: 813–821.

de Haan RL, Sheaffer CC, Samac DA, Moynihan JM, Barnes DK. 2002. Evaluation of annual *Medicago* for Upper Midwest agroecosystems. *Journal of Agronomy and Crop Science* **188**: 417–425.

Haggar RJ, Clements RO, Carr AKH, Peel S. 1984. *Crop protection handbook—grass and clover swards*, Williams RD, ed. Lavenham, UK: The Lavenham Press Ltd.

Jacquet C, Ameline-Torregrossa C, Huguet T, Krajinski F, Dumas B, Esquerre-Tugaye MT. 2005*a*. *Medicago truncatula* : a model plant to understand legume-pathogen interactions. In: *Second International Medicago workshop*, 21.

Jacquet C, Krajinski F, Ameline-Torregrossa C, Huguet T, Bottin A, Esquerre-Tugaye MT, Dumas B. 2005*b*. Characterization of the *Medicago truncatula*/*Aphanomyces euteiches* pathosystem cytological analysis and expression profiling. In: *Model Legume Congress 2005*, 107.

Johnstone GR, Barbetti MJ. 1987. Impact of fungal and virus diseases in pastures. In: Wheeler JL, Pearson CJ, Robards GE, eds. *Temperate pastures, their production, use and management*. Australian Wool Corporation Technical Publication. Melbourne: Commonwealth Scientific and Industrial Research Organization, 235–248.

Kalo P, Seres A, Taylor SA, *et al*. 2004. Comparative mapping between *Medicago sativa* and *Pisum sativum*. *Molecular Genetics and Genomics* **272**: 235–246.

Kamphuis L, Ellwood S, Oliver R. 2005. Genetic dissection of disease resistance to *Phoma* species. In: *Proceedings of the 2nd International Medicago workshop*, 52.

Kellock AW, Stubbs LL, Parbery D G. 1978. Seed-borne *Fusarium* species on subterranean clover and other pasture legumes. *Australian Journal of Agricultural Research* **29**: 975–982.

Kollmorgen JF. 1974. The pathogenicity of *Fusarium avenaceum* to wheat and legumes and its association with crop rotation. *Australian Journal of Experimental Agriculture and Animal Husbandry* **14**: 572–576.

Lamprecht SC. 1986*a*. A new disease of *Medicago truncatula* caused by *Cylindrocladium scoparium*. *Phytophylactica* **18**: 111–114.

Lamprecht SC. 1986*b*. Reaction of annual *Medicago* species to Colletotrichum crown rot caused by *Colletotrichum trifolii*. *Phytophylactica* **18**: 183–185.

Lamprecht SC, Knox-Davies PS. 1984*a*. Preliminary survey of foliage disease of annual *Medicago* spp. in South Africa. *Phytophylactica* **16**: 177–183.

Lamprecht SC, Knox-Davies PS. 1984*b*. *Colletotrichum trifolii* on annual *Medicago* spp. in South Africa. *Phytophylactica* **16**: 185–188.

Lamprecht SC, Baxter AP, Thompson AH. 1984. *Stemphylium vesicarium* on *Medicago* spp. in South Africa. *Phytophylactica* **16**: 73–75.

Lamprecht SC, Marasas WFO, Thiel PG, Schneider DJ, Knox-Davies PS. 1986. Incidence and toxigenicity of seedborne *Fusarium* species from annual *Medicago* species in South Africa. *Phytopathology* **76**: 1040–1042.

Lamprecht SC, Knox-Davies PS, Marasas WFO. 1988. Fungi associated with root rot of annual *Medicago* spp. in South Africa. *Phytophylactica* **20**: 281–286.

Lenné JM. 1994*a*. Fungal, bacterial and mycoplasma diseases of tropical pasture legumes. In: Lenné, JM, Trutman, P, eds. *Diseases of tropical pasture plants*. Wallingford, UK: CAB International, 21–160.

Lenné JM. 1994*b*. Fungal, bacterial and mycoplasma diseases of tropical pasture grasses. In: Lenné, JM, Trutman, P, eds. *Diseases of tropical pasture plants*. Wallingford, UK: CAB International, 161–196.

Lesins K, Lesins I. 1979. *Genus Medicago (Leguminosae): a taxonomic study.* The Hague: Dr. W. Junk Publishers.

Mackie JM, Lloyd DL, Ryley MJ, Irwin JAG. 1999. Fungal diseases of temperate annual pasture legumes in southern Queensland. *Australian Journal of Experimental Agriculture* **39**: 699–707.

Martinez ES, Hanson EW. 1963. Factors affecting growth, sporulation, pathogenicity and dissemination of *Leptosphaerulina briosiana*. *Phytopathology* **53**: 938–945.

Mebalds MI. 1987. Mycoflora of *Medicago truncatula, M. rugosa* and *M. littoralis* seed produced in Victoria, Australia. *Seed Science and Technology* **15**: 175–183.

Moussart A, Onfroy C, Lesné A, Esquibet M, Grenier E, Tivoli B. 2006. Host status and reaction of *Medicago truncatula* accessions to infection by three major pathogens of pea (*Pisum sativum*) and alfalfa (*Medicago sativa*). *European Journal of Plant Pathology* (in press).

Muehlbauer F, Chen W, Rajesh PN, McPhee K, Pak J, Cho S. 2005. Stalking ascochyta blight resistance in chickpea with help from model species. In: *Proceedings of the 2nd International Medicago workshop*, 16.

Neal JS, Bellotti B, Vanstone B, Graham R. 1997. Root lesion nematodes reduce annual medic growth. In: *11th Biennial Conference. Programme and Summaries*. Perth: Australasian Plant Pathology Society, 158.

Nichols PGH, Collins WJ, Barbetti MJ. 1996. Registered cultivars of subterranean clover—their origin, identification, characteristics and use in Western Australia. *Department of Agriculture, Western Australia, Bulletin* **4327.**

Nyamsuren O, Colditz F, Rosendahl S, *et al*. 2003. Transcriptional profiling of *Medicago truncatula* roots after infection with *Aphanomyces euteiches* (oomycota) identifies novel genes upregulated during this pathogenic interaction. *Physiological and Molecular Plant Pathology* **63**: 17–26.

O'Neill NR. 1996. Pathogenic variability and host resistance in the *Colletotrichum trifolii-Medicago sativa* pathosystem. *Plant Disease* **80**: 450–457.

O'Neill NR, Bauchan GR. 2000. Sources of resistance to anthracnose in the annual *Medicago* core collection. *Plant Disease* **84**: 261–267.

O'Neill NR, Bauchan GR, Samac DA. 2003. Reactions in the annual *Medicago* spp. core germ plasm collection to *Phoma medicaginis*. *Plant Disease* **87**: 557–562.

Ostazeski S, Barnes DK, Hanson CH. 1969. Laboratory selection of alfalfa for resistance to anthracnose, *Colletotrichum trifolii*. *Crop Science* **9**: 351–354.

Parmelee JA. 1962. *Uromyces striatus* Schroet. in Ontario. *Canadian Journal of Botany* **40**: 491–510.

Piano E, Francis CM. 1992. The annual species of *Medicago* in the Mediterraneane region, ecogeography and related aspects of plant introduction and breeding. In: *Proceedings of the Xth International Conference of the EUCARPIA Medicago spp Group*, 373–385.

Piano E, Pusceddu S, Spanu F. 1991. Observations on the distribution and ecology of annyual medics and subterranean clovers in Sicily (Italy). In: *Proceedings of the IV International Rangelands Congress*, 405–409.

Pilet-Nayel ML, Lesné A, Prospéri JM, Delalande M, Lecointe R, Baranger A. 2005. Resistance to A*phanomyces euteiches* in M*edicago truncatula*. In: *Model Legume Congress 2005*, 53.

Prosperi JM, Genollen V, Olivieri I, Mansat P. 1989. Observations sur la repartition et l'ecologie des luzernes annuelles et de trefle souterrain en Corse. In: *Proceedings of the XVI International Grasslands Congress*, 295–296.

Prosperi JM, Bataillon T, Delalande M, Ronfort J. 2002. The organisation of the genetic diversity in the complex of species *Medicago truncatula–Medicago littoralis*. Consequence for the efficient screening of natural allelic variation and installation of a biological center of resources. NAAIC Pre-conference *Medicago truncatula*: a Model *Medicago* Species for Molecular Biology. In: *38th North American Alfalfa Improvement Conference* (http:/www.naaic.org/Meetings/National/2002meeting/2002Abstracts/Prosperi.pdf).

Puckridge DW, French RJ. 1983. The annual legume pasture in cereal ley farming systems of southern Australia: a review. *Agriculture, Ecosystems and the Environment* **9**: 229–267.

Raynal G. 1977. Comparison, en contaminations artificielles, des pouvoirs pathogens des *Colletotrichum* isoles en France sur la Luzerne. *Annales de Phytopathologie* **9**: 193–203.

Raynal G, Gondran J, Bournoville R, Courtillot M. 1989. *Ennemis et maladies des prairies.* Paris: INRA.

Renfro BL, Sprague EW. 1959. Reaction of *Medicago* species to eight alfalfa pathogens. *Agronomy Journal* **51**: 481–483.

Robson AD. 1983. Mineral nutrition. In: Broughton, WJ, ed. *Nitrogen fixation*, Vol. 3. Oxford: Clarendon Press, 36–55.

Robson AD, Loneragan JF. 1970. Nodulation and growth of *Medicago truncatula* on acid soils. II. Colonisaton of acid soils by *Rhizobium meliloti*. *Australian Journal of Agricultural Research* **21**: 435–445.

Sampson K, Western JH. 1941. *Diseases of British grasses and herbage legumes*. London: Cambridge University Press.

Semeniuk G, Rumbaugh MD. 1976. Reaction of some perennial and annual *Medicago* species and cultivars to the yellow leafblotch disease caused by *Leptotrochila medicaginis*. *Plant Disease Reporter* **60**: 596–599.

Schmiedeknecht M. 1959. Beitrag zur eigenschaftsanalyse der resistenz verschiedener *Medicago*-Arten gegen *Pseudopeziza medicaginis* (Lib.) Sacc. *Zuchter* **29**: 65–72.

Sheaffer CC, Lake AWH. 1997. Legumes in cropping systems: approaches in Midwest United States and Southern Australia. In: Buchanan-Smith JG, Bailey LD, McCaughey P, eds. *Proceedings of the XVIII International Grassland Congress*. Calgary: Association Management Centre, 349–354.

Sheaffer CC, Simmons SR, Schmitt MA. 2001. Annual medic and berseem clover dry matter and nitrogen production in rotation with corn. *Agronomy Journal* **93**: 1080–1086.

Sheaffer CC. Gunsolus JR, Grimsbo Jewett J, Lee SH. 2002. Annual *Medicago* as a smother crop in soybean. *Journal of Agronomy and Crop Science* **188**: 408–416.

Shrestha A, Hesterman OB, Squire JM, Fisk JW, Sheaffer CC. 1998. Annual medics and Berseem clover as emergency forages. *Agronomy Journal* **90**: 197–201.

Smith JF, Jagusch KT, Brunswick LFC, Kelley RW. 1979. Coumestans in lucerne and ovulation in ewes. *New Zealand Journal of Agricultural Research* **22**: 411–416.

Thies JA, Barnes DK. 1991. Phytophthora root rot resistance. In: Fox CC, Berbert R, Gray FA, Grav CR, Jessen DL, Peterson MA, eds. *Standard tests to characterize alfalfa cultivars*, 3rd edn. Beltsville, MD: North American Alfalfa Improvement Conference, D-8.

Thomson EF, Rihawi, Cocks PS, Osman AM, Russi L. 1990. Recovery and germination rates of seeds of Mediterranean medics and clovers offered to sheep at a single meal or continuously. *Journal of Agricultural Science, Cambridge* **144**: 295–299.

Tivoli B, Lemarchand E, Moussart A, Onfroy C. 2005. What pea disease resistance studies can expect from *Medicago*? In: *Second International Medicago workshop*, 23.

Tivoli B, Baranger A, Avila C, *et al*. 2006. Screening techniques and sources of resistance to foliar diseases caused by major necrotrophic fungi in grain legumes. *Euphytica* **147**: 223–253.

Torregrossa C, Cluzet S, Fournier J, *et al*. 2004. Cytological, genetic and molecular analysis to characterize compatible and incompatible interactions between *Medicago truncatula* and *Colletotrichum trifolii*. *Molecular Plant–Microbe Interactions* **17**: 909–920.

Torregrossa C, Dumas B, Krajinski F, Esquerré-Tugaye MT, Jacquet C. 2006. Transcriptomic approaches to unravel plant-pathogen interactions in legumes. *Euphytica* **147**: 25–36.

Toyoda K, Ikeda S, Morikawa J, *et al*. 2004. A *Medicago truncatula-Mycosphaerella pinodes* interaction, a new model pathosystem for genetic dissection of susceptibility to fungal pathogen. In: *Proceedings of the 5th European Grain Legumes Conference, Transformation of Medicago truncatula via infiltration*, 318.

Trieu AT, Burleigh SH, Kardailsky IV. 2000. Transformation of seedlings or flowering plants with Agrobacterium. *Plant Journal* **22**: 531–541.

Troeung BM, Gosset H. 1990. A note on the resistance of annual medic species to anthracnose. *Fourrages.* **121**: 89–94.

Vandemark GJ, Grunwald NJ. 2004. Reaction of *Medicago truncatula* to *Aphanomyces euteiches* race 2. *Archives of Phytopathology and Plant Protection* **37**: 59–67.

Vincent JM. 1988. The role of legume, *Rhizobium*, and environments in nitrogen fixation. Constaints on symbiotic potential and their removal. In: Beck DP, Materon LA, eds. *Nitrogen fixation by legumes in Mediterranean agriculture*. Dordrecht: Martinus Nijhoff, 275–285.

Walsh MJ, Delaney RH, Groose RW, Krall JM. 2001. Performance of annual medic species (*Medicago* spp.) in Southeastern Wyoming. *Agronomy Journal* **93**: 1249–1256.

You MP, Sivasithamparam K, Riley IT, Barbetti MJ. 1999. Relationship of rainfall, cultural practices, soil and plant nutrients and seedling survival with root disease and parasitic nematode numbers in annual *Medicago* spp. pastures. *Australian Journal of Agricultural Research* **50**: 977–983.

You MP, Sivasithamparam K, Riley IT, Barbetti MJ. 2000. The occurrence of root infecting fungi and parasitic nematodes in annual *Medicago* spp. in Western Australian pastures. *Australian Journal of Agricultural Research* **51**: 435–444.

You MP, Barbetti MJ, Nichols PGH. 2005. New sources of resistance identified in *Trifolium subterraneum* breeding lines and cultivars to root rot caused by *Fusarium avenaceum* and *Pythium irregulare* and their relationship to seedling survival. *Australasian Plant Pathology* **34**: 237–244.

Zhu Y, Sheaffer CC, Barnes DK. 1996. Forage yield and quality of annual *Medicago* species in the north-central USA. *Agronomy Journal* **88**: 955–960.

Zhu Y, Sheaffer CC, Russelle MP, Vance CP. 1998. Dry matter accumulation and nitrogen fixation of annual *Medicago* species. *Agronomy Journal* **90**: 103–108.