Seed Health Aspects Involved in Seed Production of Alfalfa and Berseem in a Mediterranean Environment

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ABSTRACT

Seed samples of alfalfa (*Medicago sativa* L.) and berseem (*Trifolium alexandrinum* L.), produced in a Mediterranean environment, were tested to detect the mycoflora. Experimental field plots were established in Foggia (southern Italy) for two years (1989 and 1990) using sub-samples of the same seed sources. Fungal diseases in the field were evaluated, samples of seed were collected from each genotype and their mycoflora determined. Several fungal species, both pathogenic and apparently non-pathogenic, were observed in or on the seeds. Berseem had the largest range of fungal species. In the field, leaf damage was caused by *Stemphylium botryosum* on both crops and by *Phoma medicaginis* on alfalfa only. The results suggest an important role for seed-borne pathogenic fungi in berseem and alfalfa in a Mediterranean environment. Specific and appropriately managed seed production fields should be established for the production of healthy seed lots.

Additional index words: Medicago sativa, Trifolium alexandrinum,* seed borne pathogens, *Stemphylium botryosum, Phoma medicaginis

INTRODUCTION

The potential forage and seed yield of alfalfa (*Medicago sativa* L.) and berseem (*Trifolium alexandrinum* L.) depends on the genetic background and on the health and quality of the seed.

Regions characterised by a mild spring, dry and hot summer and rainy autumn, such as the Mediterranean favour the establishment of plant diseases, especially those caused by fungi. Many pathogens can be seed transmitted (Neergaard, 1979; Richardson, 1979) and by this means they can efficiently overwinter and spread. The use of healthy seeds is of paramount importance in order to prevent epidemics and can also contribute to improvement of forage quality and quantity (Haussmann, 1957; Graham, Frosheiser, Stuteville and Erwin, 1979).

The aims of this study were: i) to determine the seed mycoflora of commercial cultivars and ecotypes of alfalfa and berseem, and ii) to evaluate the pathogenicity of some seed-borne fungal species and their incidence in the field in a typical Mediterranean environment.

MATERIALS AND METHODS

Field experiments and plant materials

Experiments were carried out at the Experimental Institute of Forage Crops, Section of Foggia (located in southern Italy: latitude 41° 28' North and longitude 15° 33' East) in 1989 and 1990. The main climatic characteristics were described by Martiniello (1983). Samples of seed from different cultivars and ecotypes cultivated by farmers of southern Italy were used (i.e. 10 alfalfa and 32 berseem samples). Each seed sample was used for field experiments and for seed health testing at the Plant Pathology Research Institute. The cultivars, ecotypes and their origin (mostly Mediterranean basin) are shown in Table 1. Seeds from each genotype were taken at random and planted in 5 cm peat pots containing a 2:2:1 (by volume) soil-sand-peat potting mixture in a greenhouse. The pots were thinned to one plant when the seedlings were well developed and during the third week of March each year the plants were transplanted to the field. A randomised complete block design with two replications was used. Each plot was two rows spaced 50 cm apart and 1 m long, with plants spaced 5 cm within the row. At the green bud and at the late flowering stages the plants were examined and samples of leaves from genotypes showing disease symptoms were collected for further studies. At physiological maturity all the plants in the plot were harvested, threshed and a sample of 100 seeds chosen at random for evaluating seed mycoflora. The berseem seeds used in 1990 originated from the 1989 experimental plots.

Seed health testing

All seed samples were stored at 60% RH and 6 ± 1°C and assessed for the percentage of seed infected or contaminated by potentially pathogenic fungi by the blotter test (Neergaard, 1979). Fungal species considered as saprophytic were also identified. One hundred seeds per sample were incubated at 22 ± 1°C in 10 cm Petri dishes (25 per dish) under alternate cycles of 12 h NUV light (peak : 360 nm) and 12 h darkness and observed after 8 and 15 days. At the second observation any *Fusarium* spp. were recorded.

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Table 1. Alfalfa and berseem genotypes and their origin.

Genotype		Origin	Genotype		Origin
Alfalfa			**Berseem**		
Bresaola	(**)	Italy	Back Selection	(*)	Egypt
Cane	(**)	Italy	Giza 10	(**)	Egypt
Delta	(**)	Italy	LGSV	(*)	Egypt
Equipe	(**)	Italy	Multifoliate	(*)	Egypt
La Rocca	(**)	Italy	Sacromonte	(**)	Italy
Garisenda	(**)	Italy	Sakha 4	(**)	Egypt
Prospera	(**)	Italy	Synt variety	(*)	Egypt
Robot	(**)	Italy	25 ecotypes	(*)	Italy
Prosementi	(**)	Italy			
Sette	(**)	Italy			

* ecotypes; ** cultivars

Pathogenicity tests

The isolation of fungi onto Oxoid potato dextrose agar (PDA) was attempted from leaves and stems of alfalfa and berseem showing disease symptoms. Pathogenicity tests were performed with fungi isolated from seeds or diseased plants with detached leaves inoculated using a modification of the procedure described by Ward (1959). Leaves were obtained from 45 to 60 day old berseem and alfalfa plants, respectively, that had been raised in a soil-sand-peat mixture in 15 cm pots in a controlled temperature greenhouse (20 ± 3°C). Detached leaves were floated on a 2% sucrose solution in 9 cm Petri dishes and inoculated in the middle with a 15 µl drop of a suspension of conidia (10^5 ml^{-1}) obtained from a mature culture. Inoculated leaves (two replications with three leaves each) were incubated on a laboratory bench and exposed to indirect light. The temperature varied from 18 to 24°C. In the greenhouse, potted plants of the same age, kept under the same conditions described above, were inoculated by spraying with the conidial suspension until run-off. Six plants were used for each replicate.

RESULTS

Leaf spots and extended necrosis often involving the midrib and part of the leaflet blade tip were observed in the field on alfalfa but only leaf spots were present on berseem. From these lesions *Stemphylium botyrosum* Wallr. was constantly isolated. Small dark brown spots, scattered on leaflets and sometimes on petioles, were observed on alfalfa and from them *Phoma medicaginis* Malb. & Roum. (Morgan-Jones and Burch, 1987) was isolated.

The potentially pathogenic genera or species of fungi detected frequently on seed of alfalfa and berseem are shown in Tables 2 and 3 respectively. *Fusarium solani* (Mart.) Sacc. emend. Snyder & Hansen was recorded most frequently (11%) in the first year on berseem. Other *Fusarium* spp. observed on alfalfa and berseem seeds included *F. oxysporum* Schlecht. emend Snyder & Hansen, *F. equiseti* (Corda) Sacc. and *F. subglutinans* (Wollenw. & Reinking) Toussoun & Marasas. Moreover, *F. graminearum* Schwabe was also sporadically observed on berseem. Among the *Phoma* spp. detected on seeds of alfalfa and berseem, *P. glomerata* (Corda) Wollenw. & Hochapf. was the most frequently observed. Other potentially pathogenic fungi observed less frequently were *Ascochyta* spp., *Bipolaris sorokiniana* (Sacc.) Shoem., and *B. tetramera* (McKinney) Shoem. on berseem; and a *Verticillium* sp. on alfalfa. Species of fungi apparently non-pathogenic such as *Aspergillus* sp., *Aspergillus niger* v. Tieghem, *Chaetomium* sp., *Epicoccum purpurascens* Ehrenb., *Penicillium* spp., *Trichothecium roseum* (Pers.) Link were detected on seed samples of both species. *Aspergillus flavus* Link, *Curvularia* spp., *Geotrichum* sp., *Gliocladium* sp., *Helicomina* sp., *Rhizoctonia*-like fungi, and *Stachybotrys* sp. were also observed on berseem and *Mucor* sp. and *Ulocladium* spp. on alfalfa

On alfalfa the pathogenicity of *P. medicaginis* isolated from cv. Cane in 1989 in the field and from cv. Bresaola seeds (produced in 1990), was confirmed on detached leaves and on cv. Equipe plants. *S. botryosum* isolated from seeds of cv. La Rocca confirmed its pathogenicity on detached leaves of the cv. Equipe. On berseem the pathogenicity of *S. botryosum,* isolated from leaf spots on cv. Sakha 4 in 1989 and in the field was confirmed by inoculating plants of Ecotype 22, originated from southern Italy, on which symptoms similar to those observed in the field were induced.

Table 2. Major potentially pathogenic mycoflora recorded in or on original alfalfa seed source of 1988, and from the harvests of 1989 and 1990 (referred to as I, II and III respectively).

	Seed samples infected (%)			Mean % infected seeds per sample		
Fungi	**I**	**II**	**III**	**I**	**II**	**III**
Alternaria spp.	100	100	80	11 ± 7[1]	13 ± 8	7 ± 7
Cladosporium spp.	100	90	100	20 ± 7	9 ± 6	29 ± 7
Fusarium moniliforme	10	40	20	1 ± 0	4 ± 2	2 ± 1
F. solani	0	10	0	0	4 ± 0	0
Fusarium spp.	0	50	0	0	2 ± 3	0
Phoma medicaginis	0	0	10	0	0	2 ± 0
Phoma spp.	60	20	50	2 ± 1	2 ± 1	2 ± 1
Stemphylium botryosum	70	100	70	2 ± 1	6 ± 3	2 ± 1

[1]Mean ± standard deviation

Table 3. Major potentially pathogenic mycoflora recorded in or on the original berseem seed source of 1988 and from the harvests of 1989 and 1990 (referred to as I, II and III respectively)

	Seed samples infected (%)			Mean % infected seeds per sample		
Fungi	**I**	**II**	**III**	**I**	**II**	**III**
Alternaria spp.	91	100	91	14 ± 9[1]	10 ± 8	10 ± 6
Cephalosporium spp.	16	6	16	3 ± 2	1 ± 0	1 ± 0
Cladosporium spp.	75	94	100	5 ± 5	7 ± 4	8 ± 4
Fusarium moniliforme	3	25	13	1 ± 0	7 ± 6	1 ± 0
F. solani	3	6	3	11 ± 0	3 ± 3	1 ± 0
Fusarium spp.	22	66	13	4 ± 3	4 ± 4	1 ± 0
Phoma spp.	38	19	38	2 ± 2	2 ± 1	2 ± 1
Stemphylium botryosum	44	78	69	2 ± 1	3 ± 1	3 ± 2

[1]Mean ± standard deviation

DISCUSSION

Good seed health is one of the prerequisites for the full expression of the genetic characteristics of genotypes (Haussmann, 1957; Lorenzetti and Falcinelli, 1987; Papini, 1986). Seed-health testing of alfalfa and berseem showed the presence of several species of potentially pathogenic seed-borne fungi.

On berseem, species of *Alternaria*, *Cladosporium* and *Phoma* were most frequently detected on or in seed samples. The range of *Fusarium* species and their frequency was more stable in berseem than in alfalfa seed samples. However *Fusarium moniliforme* Sheldon showed a more stable seed infection rate on both forage crops. *Stemphylium botryosum* on berseem and *P. medicaginis* on alfalfa were recorded both on seed and in the field, were pathogenic and therefore may have the capability to reduce yield and quality of both these forages in the Mediterranean area, as shown by Cormack (1945), Nelson (1955) and Basu (1976) in other environments.

The comparison of the results obtained from analysing the seeds of the two forage species indicates a larger range of fungal species (including apparently non-pathogenic) on

berseem. In genotypes of both alfalfa and berseem pathogenic fungi were well represented. Several species detected from seeds, even when the percentage of infected seed is low, are potentially dangerous. In fact, seed could be an efficient way for disease transmission. Considering the high sowing rates for small seeded forages (roughly 1000 seeds per m^2), even a pathogen with a low frequency in a seed lot has a good chance to be well distributed in the field.

The overall results suggest that the use of healthy seed in these two leguminous forage crops is a focal point for producing forage and seed of good quality. Ways to obtain healthy seeds include genetic resistance and agronomic practices. Among the latter, the choice of suitable environments for seed production is of paramount importance. Chemical treatments of seed should be used with caution, considering the fact that some active ingredients can interfere with the Rhizobia-plant symbiosis. In other leguminous crops such as soybean and chickpea, some chemicals applied as seed treatments have interacted with the plant-bacterial relationship (Bhattacharya and Sengupt, 1984; Schnelle and Hensley, 1990).

In conclusion, the importance of healthy seed of leguminous crops in the Mediterranean area should be taken into account and specific seed production fields should be established (Martiniello, 1991). In this way it will be possible to apply the appropriate management for producing high-quality seed.

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