International Journal of Plant Production 2 (4), October 2008
ISSN: 1735-6814 (Print), 1735-8043 (Online)
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Seasonal dynamics and prevalence of alfalfa fungal pathogens in Zanjan province, Iran

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Received 2 March 2007; Accepted after revision 18 July 2008; Published online September 2008

Abstract

During 2000 and 2001, the prevalence of fungal diseases of alfalfa was surveyed under environmental conditions in Zanjan province, Iran. In total, 15 genera of fungal pathogens were isolated from plants sampled from the fields. All of these disease interactions had not been previously recorded in Zanjan and *Colletotrichum truncatum*, *Leptotrochila medicaginis*, *Phoma medicaginis*, *Rhizoctonia crocorum* on alfalfa were new disease reports for Iran. In 2000, the mean disease incidence of fungal pathogens of alfalfa varied by sampling time and field location. Decreases in the incidence of diseases occurred after cuttings. The incidence of diseases detected in 2000 was correlated negatively with mean monthly temperature and positively with monthly total rainfall, indicating that reductions in diseases were associated with higher temperature and less humidity, irrespective of fungal species. In 2001, *Ph. medicaginis* was the predominant fungus infected 42.8% of plants in the alfalfa fields followed by *Pseudopeziza medicaginis* (39.1%), *Uromyces striatus* (33.3%), *Peronospora trifoliorum* (29.8%), *Sporonema phacidioides* (17.7%), and *Leveillula taurica* (10.1%), irrespective of sampling time and field location. There was no significant correlation between weather variables and disease incidence for *Ph. medicaginis* or *L. taurica*. However, the incidence of common leaf spot and downy mildew were significantly correlated with both monthly mean temperature and total rainfall. The incidence of rust was positively correlated with mean monthly temperature, whereas the incidence of yellow leaf blotch was negatively correlated with monthly total rainfall. This improved knowledge of diseases of alfalfa in Zanjan will assist in the future selection of management strategies and breeding of suitable cultivars for use in regions with similar climatic conditions.

Keywords: Lucerne; foliar disease; disease incidence; weather

Introduction

Cultivated alfalfa (*Medicago sativa* L.) or lucerne was native to the Caucasus, northeastern Turkey and northwestern Iran. Alfalfa, the oldest and the most important forage crop in the world, is currently cultivated as a nitrogen source and soil-conserving

perennial crop in low-input agricultural systems (Stuteville and Erwin 1990). However, various pathogens threaten alfalfa production worldwide. Fungal diseases, such as spring black stem and leaf spot (*Phoma medicaginis* Malbr. & Roum. in Roum. var. *medicaginis* Boerema), summer black stem and leaf spot (*Cercospora medicaginis* Ellis and Everh.), common leaf spot (*Pseudopeziza medicaginis* (Lib.) Sacc.), Leptosphaerulina leaf spot (*Leptosphaerulina briosiana* (Pollacci) J. H. Graham and Luttrell), Stemphylium leaf spot (*Stemphylium* spp.) can cause severe alfalfa yield losses (Nutter et al., 2002; Wilcoxson et al., 1973). In addition to quantitative yield loss, some foliar pathogens including *Fusarium* spp. and *Alternaria* spp. (Scudamore and Livesey, 1998) can lower forage quality by producing mycotoxins. In Australia, foliar diseases reduced Medicago herbage yield by 16-20%, seed yield by 37%, and seed weight by 7-13% compared with fungicide treated plots (Barbetti, 1995). In the USA, Nutter et al. (2002) reported up to 40% yield losses to foliar diseases in alfalfa fields. Due to the lack of alfalfa resistant cultivars to most fungal pathogens (Nutter et al., 2002), fungicides and early harvest are usually recommended to minimize herbage losses. Although the effects of genotype of the host or pathogens on the development of a number of alfalfa diseases have been previously reported (Barbetti, 1987; Barbetti, 1991; Gray, 1983; Rizvi and Nutter, 1993; Tivoli et al., 2006), the effects of environmental conditions on the most foliar diseases of the crop are not clearly understood. In North Carolina, Von Chong and Campbell (1988) related the occurrence of four major alfalfa pathogens, *L. briosiana*, *Stemphylium botryosum* Wallr., *Ph. medicaginis* and *C. medicaginis*, to climatic conditions. In Iowa, Rizvi and Nutter (1993) determined correlations between weather variables (temperature and rainfall) and the occurrence of *Ph. medicaginis*, *Ps. medicaginis*, *Leptosphaerulina trifolii* (Rostr.) Petr., *C. medicaginis* and *Colletotrichum* spp., whereas there was no significant correlation between these factors for other foliar pathogens due to low frequencies of occurrence. In Columbia, the development of foliar diseases on the lower half of shoots was correlated with leaf wetness period and ambient moisture, whereas disease on the upper half of shoots was correlated only with cumulative rainfall (Emery and English, 1994).

In Iran, *Peronospora trifoliorum* de Bary (downy mildew) is one of the most important pathogens in alfalfa growing areas with cool climatic conditions and destroys 2-3 cuttings of the crop in western Iran (Behdad, 1999). Powdery mildew of alfalfa, caused by *Leveillula taurica* (Lév.) G. Arnaud (Ana. *Oidiopsis* sp.), causes significant seed and leaf loss in most parts of Iran. The other fungal pathogens, causing quantitative and qualitative losses to this crop in Iran (Behdad, 1999; Ershad, 1995), are *Ps. medicaginis*, *C. medicaginis*, *Rhizoctonia solani* Kühn (root canker) and *Colletotrichum trifolii* Bain. and Essary (anthracnose). Although climatic conditions affecting chickpea and wheat cropping have been examined (Gholipoor, 2007 a,b), the prevalence of diseases in relation to weather factors in main alfalfa growing regions in Iran is unknown. This information is crucial in determining effective management strategies and minimizing damage to the crop. Therefore, the objectives of this study were to detect various fungal pathogens associated with the crop, determine seasonal occurrence and prevalence of alfalfa diseases, and quantify relationships between the disease incidence and climatic factors in Zanjan province, Iran.

Material and methods

Field surveys

One field was selected in each of six main growing alfalfa regions of Zanjan as follows: Abhar, Eejroud, Kheirabad, Khodabandeh, Khoramdareh and Mahneshan. The fields were surveyed monthly throughout the growing season in 2000 and 2001. Mahneshan was not included in 2000 surveys. The normal growth cycle of alfalfa involves cutting the bushes approximately three times (early June, mid August and late October) per growing season from spring to early autumn (from March to October), followed by a dormant period over autumn and winter. In each visit in 2000, the number of healthy and diseased plants was counted in each of five replicate 1 m^2 areas, randomly selected in each field. In 2001, only the number of healthy and symptomatic plants infected by each of predominant pathogens, *Ph. medicaginis*, *Ps. medicaginis*, *Uromyces striatus* J. Schröt., *P. trifoliorum*, *Sporonema phacidioides* Desmaz and *L. taurica*, was recorded in 1 m^2. Disease incidence was assessed as the percentage of plants showing symptoms in each 1 m^2 area. The number of leaves or plants collected varied slightly at each site, ranging from 25 to 47 samples. Whole plant was sampled if any symptoms of wilt, basal stem or root rot, yellowing or death were observed on the plant. In 2001, 40 fallen leaves having yellow leaf blotch symptoms were also collected in each field and assessed for the presence of apothecia of *Leptotrochila medicaginis* (Fuckel) H. Schüepp (Ana. *S. phacidioides*) on lesions. Each sample was placed in a polyethylene plastic bag and transferred to the laboratory.

Symptomatic parts of each plant were rinsed in tap water, dried, surface sterilized in 1% sodium hypochlorite for 2 min, then rinsed two times in sterile distilled water. Small pieces (0.5 mm^2) were excised from the junctions of healthy and necrotic tissue of leaves, stems and roots and placed (four pieces in each 9-cm Petri dish) onto potato-dextrose agar (PDA). A minimum of 3 isolations was made from each symptom type in each sample. Plates were incubated at 22±1 with a 12 h photoperiod for up to 10 days. Single spore-derived cultures of fungi isolated from samples were prepared on PDA. For non-sporulating fungi, colonies on PDA were subcultured with hyphal tips until pure. Most of foliar pathogens, including *L. taurica*, *P. trifoliorum* and *U. striatus*, were initially diagnosed by observing typical leaf spots. The leaves were then incubated for 48-72 h on moist filter papers (Whatman no. 1; Whatman International Ltd, Maidstone, UK) in Petri dishes at room temperature to encourage sporulation on infected leaves to confirm the initial diagnosis. Both macroscopic and microscopic characteristics were used to identify the fungi, using general identification keys (Ainsworth et al., 1973; Barnett and Hunter, 1998; Ellis, 1971; Ellis, 1976; Nelson et al., 1981), specific keys (Meyer and Luttrell, 1986; Mukerji, 1968; Punithalingam and Gibson, 1972; Roberts, 1999; Laundon and Waterston, 1965) and key characteristics as described by Stuteville and Erwin (1990). Production of apothecia of *L. medicaginis* was induced using a method slightly modified from that developed by Semeniuk (1971). Apothecia formed on infected leaves placed between filter papers inside plastic mesh bags and left in outdoor conditions from autumn to mid spring. Morphological characteristics of apothecia and ascospores formed on leaves were used to identify *L. medicaginis* as described by Schuepp (1959).

Pathogenicity tests

Pathogenicity of all fungi isolated from diseased samples collected from the sites in 2000 and 2001 were assessed in the laboratory. Seeds of alfalfa cv. Hamadani were surface sterilized in 1% sodium hypochlorite for 2-3 min and washed three times in sterile water. Seeds were planted in plastic pots (10cm diam.) and then thinned to three seedlings per pot. Of four pots containing 4-week-old seedlings, three pots were inoculated with inoculum of each fungal species and one pot (control) remained noninoculated or was sprayed with sterile distilled water (regarding fungi inoculated as spore suspension). For *Alternaria* sp., *C. medicaginis*, *Colletotrichum truncatum* (Schw.) Andrus and W.D. Moore, *Ph. medicaginis*, *Stagonospora meliloti* (Lasch) Petr. (Erwin et al., 1987) and *S. botryosum*, spore suspensions from a number of PDA plates were combined and the concentration was adjusted to 5×10^6 spores/ml with the aid of a haemocytometer. For *P. trifoliorum*, symptomatic leaves were placed on moist filter papers in Petri dishes at darkness to induce sporangium production on leaf lesions. Suspensions of spores of *L. taurica* (collected from natural leaf lesions) and *P. trifoliorum* were adjusted to 10^3 spores/ml. Seedlings were sprayed with fresh spore suspensions until run-off. For *Ps. medicaginis*, a naturally infected leaf with sporulating lesions was placed on each leaf of treatment plants. Ascospore production by *L. medicaginis* on leaf lesions was induced in outdoor condition as described, and then leaves were placed on leaves of treatment plants. Each pot was covered with a plastic bag, which had been sprayed inside with water immediately after inoculation to maintain 100% relative humidity. After 72 h, the appearance of symptoms was recorded on a daily basis. Isolates of *Fusarium oxysporum* Schlechtend. ex Fr. were tested for pathogenicity by a slightly modified root dipping method (Nelson et al., 1981). *Leptosphaeria* sp. did not sporulate in culture medium and was not tested for pathogenicity. *Rhizoctonia crocorum* (Pers. ex Fr.) DC. (Naseri, 2002), *Rhizoctonia* sp. and *Phytophtora* sp. were cultured on Sand-rye grain-water medium, PDA and autoclaved moist seeds of barley, respectively, to produce inocula. These three species were tested for pathogenicity using a modified inoculum/soil mixing method (Dhingra and Sinclair, 1995). The prepared inocula for *R. crocorum* (20 g per pot), *Rhizoctonia* sp. (ten *ca* 2 mm^2 plugs per pot) and *Phytophthora* sp. (50 g per pot) were placed in the damp soil around the bases of alfalfa seedlings. Urediniospores of *U. striatus* were transferred from pustules on naturally diseased leaves to leaves of treatment plants with the aid of artist's paintbrush. Non-inoculated plants showed no symptoms.

Weather data

Daily mean air temperature and total rainfall were collected from weather stations as follows: Eejroud station (latitude 36° 25 N, longitude 48° 17E, elevation 1755 MET), Kheirabad station (36° 31 N, 48° 47 E, 1770 MET), Khodabandeh station (36° 7 N, 48° 35 E, 1887 MET), Abhar and Khoramdareh station (36° 11 N, 49° 11 E, 1575 MET), Mahneshan station (36° 46 N, 47° 40 E, 1282 MET) from April to October during 2000 and 2001. The monthly mean temperature and total rainfall were obtained from daily records by the weather stations.

Statistical analysis

Data for the incidence of diseases in alfalfa fields over the growing period in 2000 were subjected to analysis of variance (ANOVA) using GenStat version 6.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, 2002). ANOVA was also used to examine the effects of sampling time and pathogen species on the incidence of the diseases caused by six major pathogens detected in 2001 in the fields. Simple linear regression analysis (GenStat) was used to determine relationships between the incidence of alfalfa diseases surveyed in 2000 and both monthly mean temperature and total rainfall. Correlations between the incidence of each of major diseases and both monthly mean temperature and total rainfall were also examined using simple linear regression analysis.

Results

In total, 15 genera of fungal pathogens were isolated from plants sampled from alfalfa fields in Zanjan during 2000 and 2001 (Tables 1, 3). All fungi were detected in all selected fields, excepting *Alternaria* sp., *F. oxysporum*, *Leptosphaeria* sp., *Phytophthora* sp., *Rhizoctonia* sp. and *S. meliloti*, which occurred occasionally in some fields.

The two-way interaction of sampling time and field location affected (SED=3.64; df=108; $P<0.05$) mean disease incidence of fungal pathogens associated with alfalfa throughout the growing period in 2000 (Table 2). Disease incidence significantly reduced in June 2000 after the first cutting in the fields, except for Khodabandeh and Kheirabad, which were assessed before the first cutting (Table 1). Disease incidence also decreased ($P<0.05$) in August 2000 after the second cutting in Khodabandeh and Kheirabad. Abhar, Eejroud and Khoramdareh could not be sampled in August 2000.

The two-way interaction of sampling time and fungal species affected (SED=14.01; df=180; $P<0.05$) the mean disease incidence for six predominant pathogens infecting alfalfa throughout the growing period in 2001 (Figure 1). *Ph. medicaginis* was the predominant fungus infected 42.8% of alfalfa plants in the fields followed by *Ps. medicaginis* (39.1%), *U. striatus* (33.3%), *P. trifoliorum* (29.8%), *S. phacidioides* (17.7%), and *L. taurica* (10.1%), irrespective of sampling time and field location. Abhar, Eejroud and Khoramdareh in July, and Khodabandeh and Mahneshan in October could not be sampled in 2001.

In 2000 and 2001, *L. taurica* and *U. striatus* were detected on the second and third cutting crops (Table 1 and Figure 1a, f). In 2001, the mean incidence of powdery mildew in the fields peaked at 36.8% in September. The mean incidence of rust disease significantly increased from June to July and little changed to the end of the study in 2001. *Ph. medicaginis* was frequently observed on alfalfa plants only on the first and second cutting crops in 2000 (Table 1) and 2001 (Figure 1b). Furthermore, fluctuations in the mean incidence for *Ph. medicaginis* ranged from 31.2 to 84.5% over the first and second cuttings in 2001. Although *P. trifoliorum* occurred throughout the growing period, the disease was detected more frequently ($P<0.05$) on the first cutting crop than the other cuttings (Figure 1d). The mean incidence of downy mildew disease decreased from 83% in May to 0% in October 2001. *Ps. medicaginis* and *S. phacidioides* were observed from May (mid spring) onward (Figure 1c, e). The incidence of common leaf spot increased significantly from May (27.2%) to August (74.7%) and again from September (22.8%) to October (55.5%). The

greatest incidence ($P<0.05$) of yellow leaf blotch in 2001, 61.7%, was detected in September. No apothecium of *L. medicaginis* was detected on leaves held on plants or fallen leaves over the growing period and only pycnidia of *S. phacidioides* formed on leaf spots. *R. crocorum* occurred in October 2000 in Abhar and on the third cutting in 2001 in Abhar, Eejroud and Kheirabad. *C. medicaginis*, *C. truncatum* and *S. botryosum* occurred sporadically in some fields (Tables 1 and 3).

Table 1. Fungi isolated from symptomatic leaves, stems and roots of alfalfa plants sampled over growing period in 2000.

Location	Date	Fungi
Abhar	16 May	*Ph. medicaginis*, *Ps. medicaginis*, *S. phacidioides**, *P. trifoliorum*
Khoramdareh	17 May	*Ph. medicaginis*, *C. truncatum*, *S. phacidioides*, *Rhizoctonia* sp.
Kheirabad	21 May	*P. trifoliorum*, *Ph. medicaginis*, *C. truncatum*, *S. phacidioides*, *Alternaria* sp.
Khodabandeh	22 May	*P. trifoliorum*, *Ps. medicaginis*, *S. phacidioides*
Eejroud	22 May	*P. trifoliorum*, *Ps. medicaginis*, *C. truncatum*, *S. phacidioides*, *S. botryosum*
Kheirabad	1 Jun	*Ps. medicaginis*, *Ph. medicaginis*, *S. phacidioides*, *P. trifoliorum*, *F. oxysporum*, *Alternaria* sp.
Khodabandeh	7 Jun	*Ps. medicaginis*, *Ph. medicaginis*, *S. phacidioides*, *P. trifoliorum*, *C. truncatum*
Eejroud	19 Jun	*Ps. medicaginis*, *S. phacidioides*, *P. trifoliorum*, *U. striatus*
Abhar	20 Jun	*Ps. medicaginis*, *Ph. medicaginis*, *S. phacidioides*, *P. trifoliorum*
Khoramdareh	20 Jun	*Ps. medicaginis*, *Ph. medicaginis*, *S. phacidioides*, *P. trifoliorum*, *U. striatus*
Abhar	3 Jul	*Ps. medicaginis*, *Ph. medicaginis*, *S. phacidioides*, *P. trifoliorum*, *U. striatus*
Khoramdareh	3 Jul	*Ph. medicaginis*, *P. trifoliorum*, *C. truncatum*, *U. striatus*, *L. taurica*
Khodabandeh	5 Jul	*Ph. medicaginis*, *Ps. medicaginis*, *S. phacidioides*, *P. trifoliorum*, *U. striatus*
Kheirabad	17 Jul	*Ps. medicaginis*, *Ph. medicaginis*, *P. trifoliorum*, *U. striatus*, *F. oxysporum*, *U. striatus*
Eejroud	28 Jul	*Ph. medicaginis*, *Ps. medicaginis*, *S. phacidioides*, *P. trifoliorum*, *U. striatus*
Khodabandeh	18Aug†	*S. phacidioides*, *Ps. medicaginis*, *P. trifoliorum*, *L. taurica*, *S. botryosum*
Kheirabad	20 Aug	*S. phacidioides*, *Ps. medicaginis*, *L. taurica*
Khodabandeh	24 Sep	*Ps. medicaginis*, *S. phacidioides*, *U. striatus*
Eejroud	27 Sep	*Ps. medicaginis*, *S. phacidioides*, *U. striatus*
Kheirabad	27 Sep	*Ps. medicaginis*, *S. phacidioides*, *U. striatus*, *L. taurica*
Abhar	28 Sep	*Ps. medicaginis*, *S. phacidioides*, *U. striatus*, *L. taurica, C. truncatum*, *C. medicaginis*
Khoramdareh	28 Sep	*Ps. medicaginis*, *S. phacidioides*, *U. striatus*, *C. medicaginis*
Abhar	22 Oct	*Ps. medicaginis*, *P. trifoliorum*, *S. phacidioides*, *U. striatus*, *L. taurica*
Khoramdareh	22 Oct	*Ps. medicaginis*, *S. phacidioides*, *U. striatus*, *S. botryosum*
Kheirabad	23 Oct	*Ps. medicaginis*, *P. trifoliorum*, *U. striatus*, *L. taurica, S. phacidioides*, *C. medicaginis*
Khodabandeh	25 Oct	*Ps. medicaginis*, *P. trifoliorum*, *S. phacidioides*, *U. striatus*
Eejroud	25 Oct	*Ps. medicaginis*, *U. striatus*, *R. crocorum*

**Leptotrochila medicaginis* (Ana. *Sporonema phacidioides*) formed on leaves after harvest and only pycnidia were evident on leaf lesions during growing period.

† Abhar, Eejroud and Khoramdareh were not sampled in August.

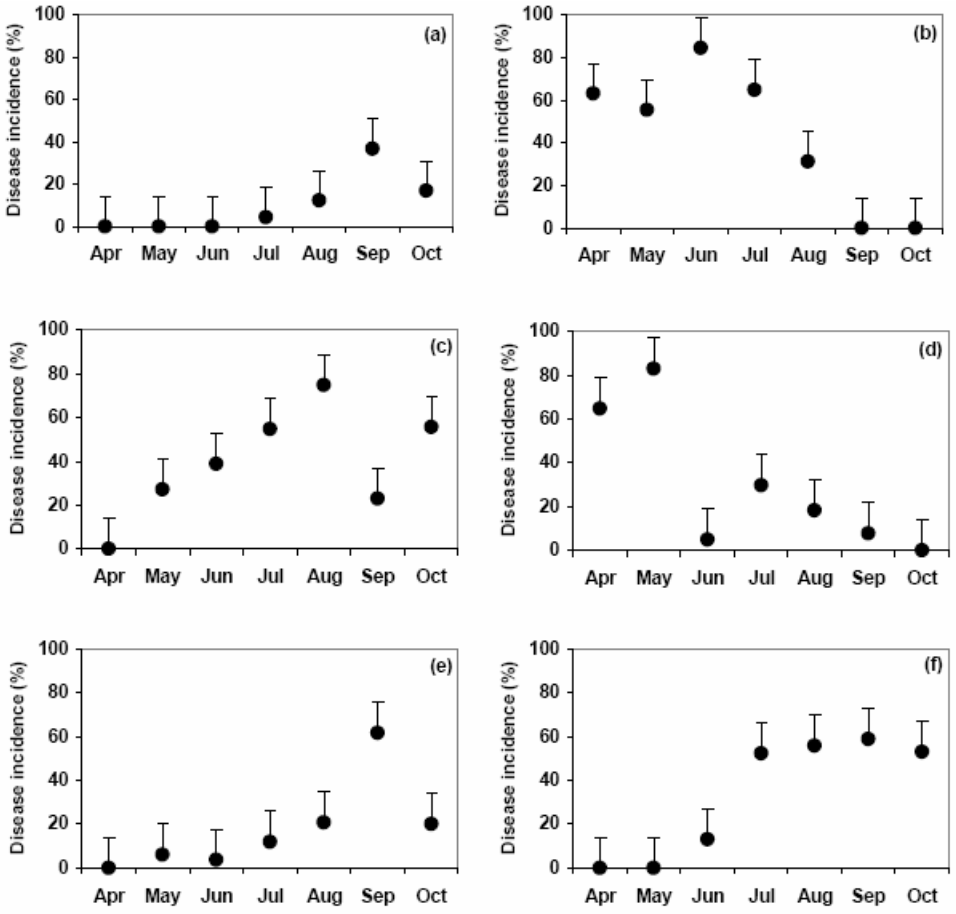

Figure 1. Disease incidence (mean percentage symptomatic plants in five replicate 1 m^2 areas per field) for predominant fungal pathogens in alfalfa fields surveyed in 2001; (a) *Leveillula taurica*, (b) *Phoma medicaginis*, (c) *Pseudopeziza medicaginis*, (d) *Peronospora trifoliorum*, (e) *Sporonema phacidioides* and (f) *Uromyces striatus*; SED (vertical bars) = 14.01; df=180; $P<0.05$.

Weather data

Over the growing period in most locations, the least monthly total rainfall coincided with the highest mean monthly temperature in August 2000 and 2001 (Figure 2). Warm weather in June also coincided with no rainfall in 2000 and $0 \leq$ rainfall ≥ 2 mm in 2001. During 2000 and 2001, total monthly rainfall increased in July and October in most locations. Each month, the highest mean monthly temperature was recorded in Khoramdareh in 2000 and Mahneshan in 2001, with lowest ones being recorded in Kheirabad (Figure 2).

Table 2. Incidence of alfalfa diseases (mean percentage symptomatic plants in five replicate 1m^2 areas per field) over growing period in 2000; SED=3.64; df =108; P<0.05.

Date	Location				
	Abhar	Eejroud	Kheirabad	Khodabandeh	Khoramdareh
May	82.2	77.2	79.2	85.6	85.8
Jun	38.2	41.8	88	87	40.6
Jul	69.6	83	86	68.8	83
Aug	-*	-	41.6	38.4	-
Sep	83.2	80	79.8	79.6	82
Oct	81.6	81.8	83.2	80.6	81.2

*Abhar, Eejroud and Khoramdareh were not sampled in August.

In 2000, the incidence of fungal diseases in the fields surveyed was significantly correlated (P<0.05) with both mean monthly temperature and total rainfall. Regression coefficients between the incidence and both mean monthly temperature and total rainfall were -1.442 (P=0.046) and 0.787 (P=0.036), respectively. In 2001, the incidence of diseases caused by *P. trifoliorum* and *Ps. medicaginis* in the fields were significantly correlated with both mean monthly temperature and total rainfall (Table 4). In contrast, there was no significant relationship between the incidence of either *L. taurica* or *Ph. medicaginis* and weather variables. The incidence of rust and yellow leaf blotch was correlated with mean monthly temperature and total rainfall, respectively (Table 4).

Discussion

These experiments provide detailed information on the prevalence and seasonal patterns of various diseases on alfalfa in fields surveyed in Zanjan, Iran. All of alfalfa diseases detected in this study had not been previously recorded in Zanjan and *C. truncatum*, *L. medicaginis*, *Ph. medicaginis* and *R. crocorum* on alfalfa were new reports for Iran. The occurrence of *R. crocorum* on alfalfa was preliminary reported (Naseri, 2002). In general, the mean disease incidence of pathogens frequently isolated from alfalfa plants in 2001 varied by sampling time and fungal species. *Ph. medicaginis* was the most common pathogen infected 42.8% of alfalfa plants in the fields followed by *Ps. medicaginis*, *U. striatus*, *P. trifoliorum*, *S. phacidioides* and *L. taurica*. In Queensland, Australia (Mackie et al., 1999), rust and in Eastern Europe (Bocsa et al., 1994), Verticillium and Fusarium diseases were the dominant diseases on alfalfa. In Iowa, *Ph. medicaginis*, *L. trifolii*, *Ps. medicaginis* and *C. medicaginis* occurred in the highest frequencies (Rizvi and Nutter, 1993). In Wyoming (Gray, 1983), *Ps. medicaginis*, *P. trifoliorum* and *Ph. medicaginis* predominated on irrigated alfalfa, whereas *L. medicaginis* was the major foliar disease of the dryland crop. Differences in environmental conditions and in the genotype of the host or pathogens in these regions may have contributed to the differences in the prevalence and complex of pathogens on alfalfa. In addition, interactions between various pathogens simultaneously present on alfalfa plants might have affected the development of diseases on the crop that need further investigation.

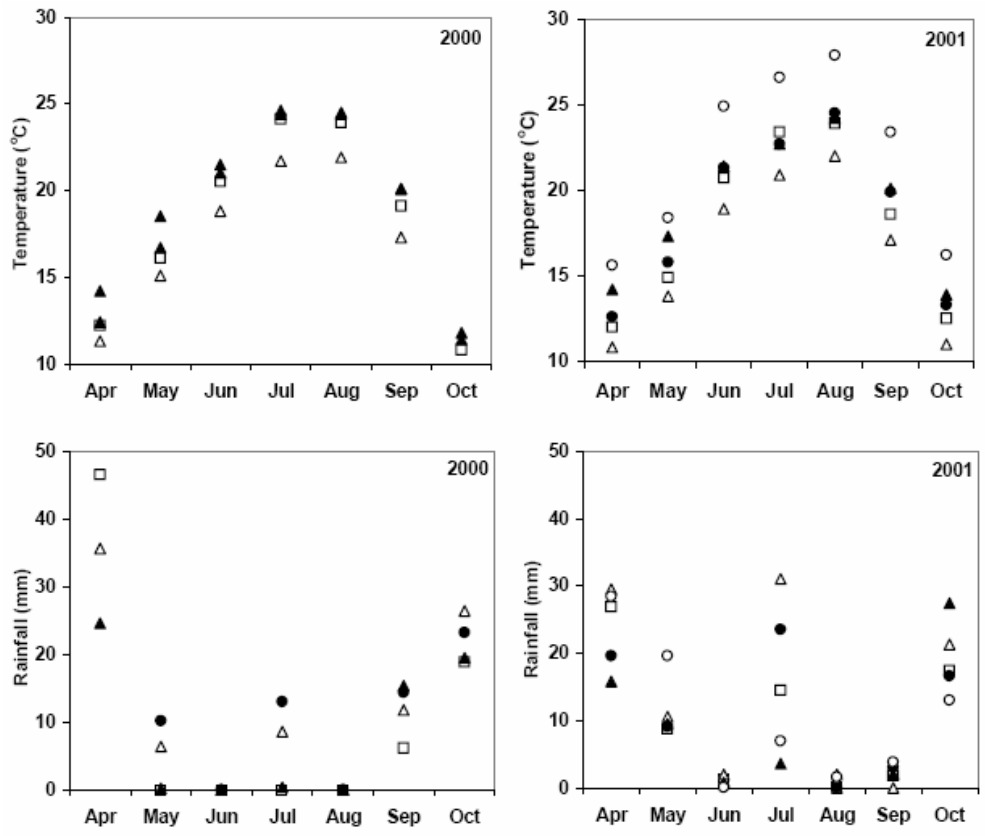

Figure 2. Monthly mean temperature and total rainfall during alfalfa growing period in Eejroud □, Khodabandeh ●, Khoramdareh ▲, Kheirabad Δ, Mahneshan ○, in 2000 and 2001.

Results are in agreement with findings in North America, Europe, South Africa and other temperate regions where spring black stem and leaf spot is a major destructive disease in perennial alfalfa (O'Neill et al., 2003). Results also parallel the seasonal patterns of *Ph. medicaginis* observed in Wyoming (Gray, 1983) and Iowa (Rizvi and Nutter, 1993), in that the disease was most frequently present in the spring and decreased as the summer progressed. Although the occurrence of the pathogen was negatively correlated with temperature variable in Iowa (Rizvi and Nutter, 1993), there was no significant correlation between weather and disease incidence examined in this study. Effects of environmental factors on *Ph. medicaginis* have also been reported elsewhere. In Australia, the severity and infection rate of *Ph. medicaginis* on *Medicago* spp. increased by extending the duration of wetness and was greater at 21/16°C than 15/10°C in the controlled environment (Barbetti, 1987). In addition, *Ph. medicaginis* produced more conidia on PDA at 23°C than at 30°C (Chung and Wilcoxson, 1971). Favorable mean monthly temperatures (19-25°C), nearly close to optimal temperatures for the pathogen (Barbetti, 1987; Chung and

Table 3. Fungi isolated from symptomatic leaves, stems and roots of alfalfa plants sampled over growing period in 2001.

Location	Date	Fungi
Mahneshan	11 Apr	*P. trifoliorum*, *Ph. medicaginis*
Khodabandeh	11 Apr	*P. trifoliorum*, *Ph. medicaginis*, *S. botryosum*
Abhar	14 Apr	*P. trifoliorum*, *Ph. medicaginis*, *Phytophthora* sp.
Khoramdareh	14 Apr	*P. trifoliorum*, *Ph. medicaginis*
Eejroud	25 Apr	*Ph. medicaginis*, *P. trifoliorum*, *Phytophthora* sp., *S. botryosum*
Kheirabad	26 Apr	*Ph. medicaginis*, *C. truncatum*, *Leptosphaeria* sp., *Rhizoctonia* sp.
Khoramdareh	3 May	*Ph. medicaginis*, *P. trifoliorum*, *C. truncatum*
Khodabandeh	3 May	*Ph. medicaginis*, *P. trifoliorum*, *C. truncatum*, *S. meliloti*
Abhar	3 May	*P. trifoliorum*, *Ps. medicaginis*, *C. truncatum*, *Leptosphaeria* sp.
Mahneshan	29 May	*P. trifoliorum*, *Ps. medicaginis*, *C. truncatum*
Kheirabad	30 May	*Ph. medicaginis*, *P. trifoliorum*, *Ps. medicaginis*, *C. truncatum*, *Leptosphaeria* sp., *S. phacidioides**, *S. botryosum*
Eejroud	31 May	*Ps. medicaginis*, *Ph. medicaginis*, *P. trifoliorum* (83)
Abhar	25 Jun	*Ps. medicaginis*, *Ph. medicaginis*, *S. phacidioides*, *P. trifoliorum*, *C. truncatum*
Khoramdareh	25 Jun	*Ps. medicaginis*, *Ph. medicaginis*, *S. phacidioides*, *P. trifoliorum*, *C. truncatum*
Khodabandeh	25 Jun	*Ps. medicaginis*, *Ph. medicaginis*, *S. phacidioides*, *P. trifoliorum*, *C. truncatum*
Kheirabad	25 Jun	*Ps. medicaginis*, *Ph. medicaginis*, *S. phacidioides*, *P. trifoliorum*, *C. truncatum*, *U. striatus*
Mahneshan	26 Jun	*Ph. medicaginis*, *P. trifoliorum*, *U. striatus*
Eejroud	26 Jun	*Ph. medicaginis*, *Ps. medicaginis*, *S. phacidioides*, *P. trifoliorum*, *U. striatus*
Khodabandeh	2 Jul†	*Ph. medicaginis*, *Ps. medicaginis*, *S. phacidioides*, *P. trifoliorum*, *U. striatus*
Mahneshan	8 Jul	*Ps. medicaginis*, *P. trifoliorum*, *U. striatus*, *L. taurica*
Kheirabad	30 Jul	*P. trifoliorum*, *Ph. medicaginis*, *U. striatus*, *L. taurica*
Mahneshan	8 Aug	*Ps. medicaginis*, *P. trifoliorum*, *U. striatus*
Khodabandeh	9 Aug	*Ph. medicaginis*, *Ps. medicaginis*, *P. trifoliorum*, *S. meliloti*
Eejroud	12 Aug	*Ps. medicaginis*, *Ph. medicaginis*, *C. truncatum*, *U. striatus*
Abhar	12 Aug	*Ps. medicaginis*, *U. striatus*, *Leptosphaeria* sp., *C. medicaginis*
Khoramdareh	16 Aug	*Ps. medicaginis*, *P. trifoliorum*, *S. phacidioides*, *U. striatus*, *L. taurica*, *S. botryosum*
Kheirabad	29 Aug	*Ps. medicaginis*, *S. phacidioides*, *U. striatus*, *L. taurica, R. crocorum*
Kheirabad	13 Sep	*Ps. medicaginis*, *S. phacidioides*, *U. striatus*, *L. taurica, C. truncatum*, *C. medicaginis*
Eejroud	17 Sep	*P. trifoliorum*, *S. phacidioides*, *U. striatus*, *C. medicaginis*
Mahneshan	17 Sep	*S. phacidioides*, *U. striatus*, *C. medicaginis*
Khodabandeh	18 Sep	*P. trifoliorum*, *S. phacidioides*, *U. striatus*, *L. taurica*
Abhar	19 Sep	*U. striatus*, *L. taurica, R. crocorum*, *C. medicaginis*
Khoramdareh	19 Sep	*Ps. medicaginis*, *S. phacidioides*, *U. striatus*, *C. medicaginis*
Abhar	1 Oct[2]	*U. striatus*, *L. taurica, R. crocorum*
Khoramdareh	1 Oct	*Ps. medicaginis*, *S. phacidioides*, *U. striatus*, *C. medicaginis*
Kheirabad	11 Oct	*Ps. medicaginis*, *R. crocorum*
Eejroud	11 Oct	*Ps. medicaginis*, *U. striatus*, *R. crocorum*

* *Leptotrochila medicaginis* (Ana. *Sporonema phacidioides*) formed on leaves after harvest and only pycnidia were evident on leaf lesions during growing period.

† Abhar, Eejroud and Khoramdareh in July, and Khodabandeh and Mahneshan in October were not sampled.

Wilcoxson, 1971), may be partially responsible for the peak of disease incidence in June. Results also differ from findings in Iowa, in that the disease was observed in July 2001 (early summer) as frequently as that in spring in the present research. This might be due to the increase of rainfall in July in most locations; however, the effect of rainfall was not consistent during the survey. On the other hands, the ranges of monthly mean temperature and total rainfall in April were similar to those in October 2001, whereas mean incidence was significantly greater in April (63.2%) than October (0%). Therefore, factors other than temperature and rainfall may have restricted the development of the disease on the last cutting crop in this study and identification of such factors merits further research.

In the present research, *Ps. medicaginis* was the second predominant pathogen on alfalfa after *Ph. medicaginis*. Although the disease incidence of *Ps. medicaginis* decreased during dry and warm summers in California (Summers and McClellan, 1975), the incidence of common leaf spot increased over summer in this study that is in agreement with seasonal patterns of the disease in Iowa (Rizvi and Nutter, 1993). Although, the occurrence of common leaf spot was correlated only with temperature in Iowa (Rizvi and Nutter, 1993), the disease incidence was correlated positively with mean monthly temperature and negatively with monthly total rainfall in this study. Therefore, highest temperature and least rainfall in the regions may be responsible for the increase of common leaf spot to the greatest level in August 2001. These relations may contribute to the future prediction of the disease progress in alfalfa fields under conditions similar to those encountered in the present research.

Table 4. Correlation coefficients between the means of incidence of the predominant diseases during growing period in alfalfa fields surveyed in 2001 and both monthly mean temperature (T) and total rainfall (R).

Disease	Weather variable	Regression coefficient	Prob>F
Powdery mildew	T	0.126	0.883^{NS}
	R	- 0.319	0.396^{NS}
Spring black stem and leaf spot	T	- 0.72	0.648^{NS}
	R	0.291	0.674^{NS}
Common leaf spot	T	2.9	0.014^{**}
	R	- 0.992	0.062^{*}
Downy mildew	T	- 2.54	0.038^{**}
	R	0.939	0.084^{*}
Yellow leaf blotch	T	0.91	0.404^{NS}
	R	- 0.766	0.105^{*}
Rust	T	2.79	0.016^{**}
	R	- 0.501	0.34^{NS}

^{NS}=not significant; ** =P≤ 0.05; * = P≤ 0.10.

In this study, the incidence of rust, the third most common disease, was positively correlated with mean monthly temperature. This may explain why *U. striatus* occurred frequently in summer, when mean monthly temperature ranged from 17.1 to 27.9°C in the regions, and slightly decreased with decreasing temperature (11-16.2°C) in October. Results agree with seasonal patterns in Australia (Mackie et al., 1999) that rust was more prevalent in warmer months. In Iowa, Webb and Nutter (1997) also found that increasing

temperatures from 15 to 30°C reduced the latent period (time from inoculation to 50% pustules appearance) and increased the rate of pustule appearance, whereas infection efficiencies (the number of pustules in 1 cm^2 leaf area) were higher at 17.5 and 19°C. In this study, mean monthly temperatures over summer in different regions were within the range of temperature optima (Webb and Nutter, 1997) for the disease development on alfalfa. Although temperatures in April and May were similar to or even higher than those in October, no rust disease was evident before the first cutting. Further investigation may explain the basis for the absence of rust in spring.

The results support previous findings (Stuteville and Erwin, 1990) that *P. trifoliorum* is generally more prevalent on the first cutting crop. It is known that conidia of *P. trifoliorum* are produced under near 100% relative humidity and germinate only in free water at 4-29°C (Stuteville and Erwin, 1990). In the present research, *P. trifoliorum* occurred more frequently over April and May 2001, when mean monthly temperature and total rainfall in the locations ranged 10.8-18.4°C and 8.7-29.5 mm, respectively. This suggests that appropriate weather conditions in April-May induced infection and sporulation by the pathogen on alfalfa. Regression analysis also demonstrated that the incidence of downy mildew was correlated negatively with mean monthly temperature and positively with monthly total rainfall, indicating that relatively higher incidence of the disease was associated with monthly lower temperature and greater rainfall. Such information may contribute to the development of forecasting models and optimization of management strategies to control downy mildew.

No apothecium of *L. medicaginis* was detected on leaves held on plants or leaves on the ground over the growing period; however, yellow leaf blotch was observed from May (mid spring) onward. As ascospores are the only known pathogenic inoculum of *L. medicaginis* (Stuteville and Erwin, 1990), ascospore must have been formed on lesions and discharged from fallen leaves on the ground throughout alfalfa growing period. Ascospore release and germination, and infection by *L. medicaginis* are favored by temperatures at < 25, 7.5-21 and 20°C (Stuteville and Erwin, 1990), respectively, and require relative humidities above 97% (Semeniuk, 1993). Mean daily temperatures over May-October in the regions ranged from 3.1 to 36.4°C, while daily minimum temperatures during this time were below 19.3°C (Zanjan Meteorological Office, 2007). In addition, there was no or negligible rainfall in June, August and September. Therefore, it was during the nighttime hours when the most favorable temperatures and extended dew periods for sporulation and infection were most likely to coincide. Furthermore, the incidence of yellow leaf blotch was correlated negatively with monthly total rainfall, suggesting that low rainfall (0-3.8 mm) in the regions may have resulted in the significant increase of yellow leaf blotch in September 2001. Results agree with earlier findings in Wyoming (Gray, 1983) and France (Leyronas et al., 2004) where *L. medicaginis* occurred primarily on alfalfa under dry and hot climate. Although rainfalls in June and August were as low as those in September, relatively more appropriate temperatures (mostly < 21°C) for *L. medicaginis* were likely responsible for significantly greater incidence of the disease in September than that in June or August.

Most of earlier studies have evaluated the severity rating or isolation frequency of fungal pathogens on alfalfa. A few studies have examined the incidence of fungal diseases on the crop; however, relationships between the incidence of alfalfa diseases and weather factors are poorly understood. The present research provides information on the overall

incidence of fungal diseases and the incidence of each major disease on alfalfa over the growing season in the fields surveyed. It also adds to our knowledge that the incidence of alfalfa diseases detected in 2000 was correlated negatively with mean monthly temperature and positively with monthly total rainfall, suggesting that regardless of fungal species, higher temperature and less humidity resulted in less disease. Furthermore, the incidence of four major alfalfa diseases, common leaf spot, downy mildew, yellow leaf blotch and rust, were correlated with weather variables. Such information concerning the relative importance of alfalfa diseases and their seasonal patterns would facilitate prioritization of research needs and the development of effective management programs.

Acknowledgements

This study was supported by the grant from the Iranian Ministry of Agriculture, research project no. 111-12-79-066. The authors thank Mr MR Moini for supporting field visits and Mr M Maleki for technical assistance throughout the survey.

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