The occurrence of barley root diseases in different agri-ecological zones of Syria

J.A.G. van Leur and K.L. Bailey

Abstract: A survey was conducted to determine the occurrence of root diseases and the identity of causal agents on barley grown in northern agricultural areas of Syria in 1992 and 1993. In 1992, 20 plants with at least 1 cm long subcrown internodes were randomly selected from each of 53 fields when plants were between flowering and maturity. In 1993, 50 plants were selected from each of 72 fields. In addtion, four wheat fields were sampled in 1993. Plants were visually rated for disease severity on the subcrown internode. Fungal cultures were isolated from root tissues by plating on selective and general media. Isolates were tested for pathogenicity using a seedling bioassay, and representative pathogens were identified to the species level. Root rot was more severe in the drier agricultural zones and disease severity increased with crop maturity. There was a weak negative association between disease severity and the number of tillers per plant. The most prevalent pathogens in both years were *Microdochium nivale* (average of 43%), *Cochliobolus sativus* (35%), red-pigmented *Fusaria* (13%), and *Microdochium bolleyi* (8%). More than 80% of the *C. sativus* and *M. nivale* isolates, and less than 20% of the *Fusarium* spp. and *M. bolleyi* isolates, were pathogenic. *Cochliobolus sativus* was most common in the drier areas, whereas *M. nivale* was most common in the wetter areas, particularly from the northwest region. This paper is the first report of *M. nivale* and *M. bolleyi* on barley and *Gaeumannomyces graminis* var. *tritici* on wheat in Syria. Other root disorders (pests) found included cyst-forming nematodes and *Porphyrophora tritici*.

Key words: barley, root rot, survey.

Résumé : En 1992 et 1993, un relevé des maladies de racine chez l'orge a été effectué dans les régions agricoles du nord de la Syrie pour en connaître la fréquence et l'identité des agents pathogènes en cause. En 1992, 20 plantes ayant un entre-noeud sub-coronal d'au moins 1 cm de long ont été choisies au hasard, entre la floraison et la maturité, dans chacun de 53 champs. En 1993, 50 plantes ont été choisies dans chacun de 72 champs. De plus, quatre champs de blé ont été échantillonnés en 1993. L'intensité des maladies a été évaluée visuellement sur l'entre-nœud sub-coronal. Des cultures fongiques ont été obtenues à partir des tissus de racines par isolement sur des milieux sélectifs ou généraux. Le pouvoir pathogène des isolats a été établi par essai biologique sur des semis et l'identité des champignons pathogènes représentatifs a été établie à l'espèce. La pourriture de racine était plus intense dans les zones agricoles plus sèches et la gravité des maladies augmentait avec la maturité de la culture. Une faible relation négative a été trouvée entre la gravité des maladies et le nombre de talles par plante. Les agents pathogènes les plus fréquents lors des deux années ont été le *Microdochium nivale* (moyenne de 43%), le *Cochliobolus sativus* (35%), les fusariums carmin (13%) et le *Microdochium bolleyi* (8%). Plus de 80% des isolats du *C. sativus* et du *M. nivale*, et moins de 20% des isolats des *Fusarium* spp. et du *M. bolleyi*, étaient pathogènes. Le *C. sativus* était plus commun dans les régions plus sèches alors que le *M. nivale* était plus commun dans les régions plus humides, particulièrement dans le nord-ouest. Le présent rapport est le premier sur la présence du *M. nivale* et du *M. bolleyi* sur l'orge et du *Gaeumannomyces graminis* var. *tritici* sur le blé en Syrie. Le nématode à kyste et le *Porphyrophora tritici* font partie des autres ravageurs qui ont été trouvés.

Mots clés : orge, pourriture de racine, relevé.

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J.A.G. van Leur.[1] The International Center for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria.
K.L. Bailey.[2] Agriculture and Agri-Food Canada, Research Centre, 107 Science Place, Saskatoon, SK S7N 0X2, Canada.

[1]Present address: NSW Agriculture, Tamworth Centre for Crop Improvement, RMB 944, Calala Lane, Tamworth NSW 2340, Australia.
[2]Corresponding author (e-mail: baileyk@em.agr.ca).

Introduction

Cereal production in the moisture-limited environments of Syria and other countries in western Asia and North Africa is dominated by barley. Barley is usually grown with minimum inputs for animal feed. In Syria, rainfall is adequate for dryland cereal cultivation in the northern and western parts, but mean precipitation becomes progressively lower in the south and central regions. Rain falls almost exclusively during the period from November to May. Temperatures are relatively mild in the coastal areas, but extremes

occur inland, with periods of frosts common in most of the cereal-growing areas.

In the last 25 years, the area of barley production in Syria has increased from 7×10^5 to 1.5×10^6 ha but yields dropped from 646 to 505 kg·ha^{-1} (Anonymous 1990). The barley acreage has increased partly because the traditional fallow system has been replaced by continuous cereal cultivation and partly because cultivation has extended into rangelands, which have unfavorable and drought-stressed environments. Jones and Singh (1995) showed that continuous barley cultivation in this region resulted in a yield decline that cannot be compensated for by the addition of fertilizer. It is not yet known to what extent root diseases (and pests) have a role in this decline but research in Canada, the United States, and Australia have demonstrated an increase of root diseases, especially common root rot, under continuous cereal cultivation (Piening and Orr 1988; Conner and Atkinson 1989; El-Nashaar and Stack 1989; Wildermuth and McNamara 1991; Bailey et al. 1992).

The term common root rot is used to describe a complex of fungal root pathogens in cereals, composed of *Cochliobolus sativus* (Ito & Kurib.) Drechsl. ex Dastur (anamorph *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem.), and *Fusarium* spp., particularly *Fusarium culmorum* (W.G. Sm.) Sacc. and *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schw.) Petch), which cause necrosis and rot of basal stems, crown and subcrown internodes (Tinline et al. 1975, 1991). *Cochliobolus sativus* and *F. culmorum* are mainly responsible for causing common root rot on barley in the Canadian prairies, Great Plains of the United States, and Russia, whereas *F. culmorum* and *F. graminearum*, which cause crown and foot rot, are more common in the Pacific Northwest of the United States and the Mediterranean area of the Middle East (Duczek and Piening 1982; Mathre 1982). Fouly et al. (1996) reported that *F. graminearum* and *Helminthosporium* spp. were also aggressive root pathogens of wheat in Egypt.

Microdochium nivale (Ces. ex Berl. & Vogl.) Samuels & Hallett (syns. *Gerlachia nivalis* (Ces. ex Berl. & Vogl.) E. Müller) causes pink snow mold of winter barley, winter wheat, grasses, and other cereals, as well as being one of several causal agents in fusarium head blight of small grains and corn (Mathre 1982). This pathogen causes diseased wheat roots and lower leaf sheaths in areas with a prolonged snow cover (Bruehl 1982). In Canada, *M. nivale* is rarely isolated from cereal seed samples (Gordon 1952; Clear and Patrick 1993), although it is frequently observed on winter cereals in Saskatchewan (Gossen and Reiter 1989). In the Turkish Central Anatolian Plateau, the most prevalent root pathogens on wheat and barley were *M. nivale* and *C. sativus* (Mamluk et al. 1997). *Microdochium nivale* has not been previously described in Syria (Mamluk et al. 1992), although weather data collected from the International Centre of Agricultural Research in the Dry Areas in Tel Hadya, Syria, show an average of 77 d per growing season with soil temperatures between 0°C and 10°C, a range suitable for the growth of the pathogen. Studies in England have demonstrated that *M. nivale* can reduce stand and tiller number of wheat plants in cold, dry soils, even without snow cover (Bateman 1993). Harris (1986) from South Australia describes its importance as a barley root pathogen in relatively mild climates.

Root diseases have been identified as limiting factors in dryland barley production systems (Mathre 1982). Some, like common root rot, tend to be more severe under moisture-limited conditions (Piening et al. 1976; Bailey et al. 1989). Therefore, a study of root diseases is of special importance to barley-growing environments where moisture stress is frequent. Little research on barley root diseases has been carried out in Syria, even though *C. sativus* was the most frequently isolated pathogen from barley subcrown internodes at one experimental site (van Leur et al. 1991). This pathogen caused up to 30% yield loss in some cultivars grown in artificially inoculated field plots (van Leur et al. 1997). No systematic surveys for barley root rots in this area have been reported. This study was undertaken to record the occurrence and identity of root diseases on barley in different agri-ecological zones in Syria.

Materials and methods

Plant sampling and disease scoring

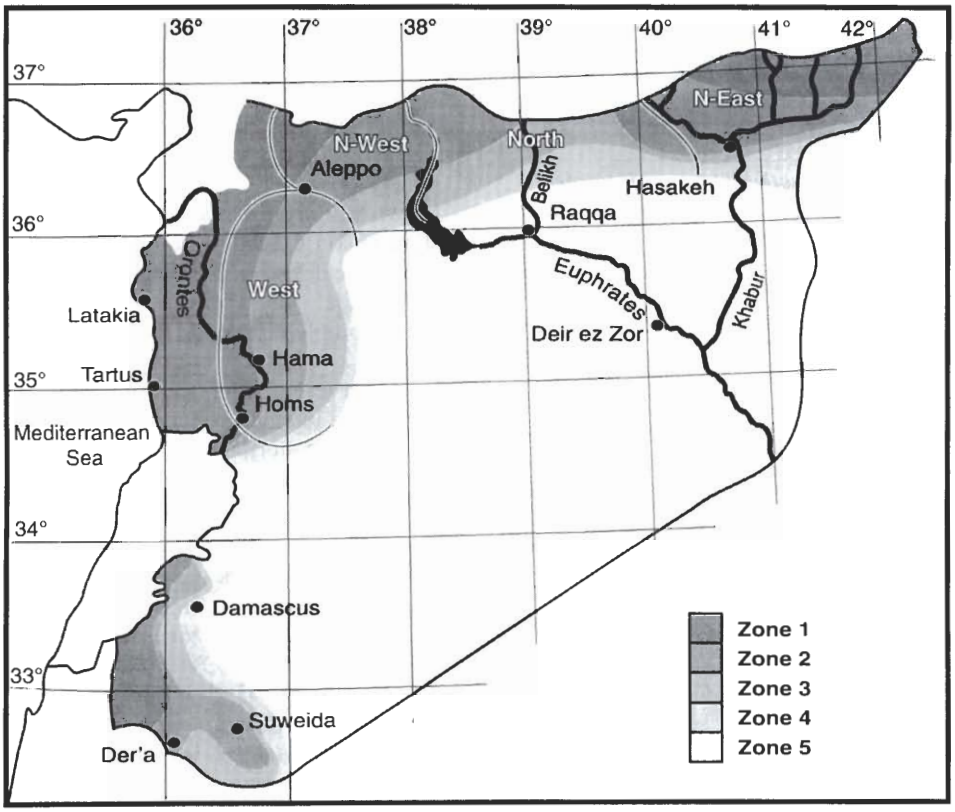
Surveys were conducted when most barley plants were past flowering (milk to soft dough stages), from late April through May in 1992 (53 fields) and 1993 (72 fields). Fields were situated near roads, but otherwise chosen at random. We sampled plants randomly while walking a diagonal path across the field.

Locations were categorized in two ways: agricultural stability zones that distinguish rainfall and cropping patterns (Anonymous 1990) and geographical regions in Syria (west, northwest, north, and northeast, Fig. 1). Agricultural stability zone 1 typically has average annual rainfall greater than 350 mm, but not less than 300 mm. The main crops are wheat, pulses, and summer crops. For the purposes of this study, irrigated fields were classed as zone 1 with regard to moisture level. Zone 2 has average annual rainfall between 250 and 300 mm, growing mainly barley, wheat, pulses, and summer crops. Zone 3 grows mainly barley with precipitation about 250 mm. Zone 4 also grows mainly barley but is drier, with rainfall between 200 and 250 mm annually. Zone 5 has rainfall less than 200 mm and is either rangeland or not cultivated; these areas were not surveyed.

Crop development (Zadoks et al. 1974), plant height, and general appearance of the crop were recorded. The presence of nematode cysts on roots was noted as well. Barley was classified as 6-row (exotic cultivars) and white or black seeded 2-row (land race) types. In 1993, 12 different fields in the northeast region were sampled twice; first on 25 and 26 April at growth stages 58–73 and again on 23 and 24 May at plant maturity (growth stages 87–93). Data from these 12 fields were used to examine changes in the isolation of fungi over time. Also in 1993, four fields of wheat bordering barley fields were sampled.

In 1992, root rot symptoms were scored on a random sample of 20 plants per field, with each plant having a subcrown internode length of at least 1 cm. Plants with subcrown internodes less than 1 cm were discarded during the sampling process. In 1993, the sample size was in-

Fig. 1. Map of agricultural stability zones and four geographical regions (west, northwest, north, and northeast) in Syria.



Source: Climatic Atlas of Syria(1977) and The Annual Agricultural Statistical Abstract(1990).

creased to 50 plants. Plants were cut 10 cm above the crown, and the root portion was stored loosely in paper bags. Samples were washed, dried at room temperature, scored for root rot symptoms, and stored dry at 5°C until fungal isolation.

A subcrown-internode score (SIS) was used, based on the disease rating scale of Wildermuth (1986), but modified to accommodate differences in intensity as well as in severity of discolouration on the subcrown internode: 0 = no symptoms; 1 = <10% light brown lesions, <1% black lesions; 2 = 10–50% light brown lesions, 1–10% black lesions; 3 = >50% light brown lesions, 10–50% black lesions; 4 = 50–99% black lesions; 5 = completely covered with black lesions. To correlate tiller production with SIS, the number of tillers per plant was counted. For other analyses, the average SIS was calculated for each field. The percentage of diseased plants (PDP) in a field was a measure of the number of plants in the sample with greater than 50% lesions on the subcrown internode (i.e., SIS ≥ 3).

Fungal isolation

Samples from each field were divided into three subsamples based on SIS: low (SIS 0–1), medium (SIS 2–3), and high (SIS 4–5). The subsamples were handled separately during the isolation process. In 1992, all 20 plants were used for isolation, but in 1993, only five plants per subsample were used. Since all plants were not used in 1993, a weighted isolation frequency per field was calculated, using the frequency of the three subsamples in the total sample.

Plants were cut just above and 1 cm below the crown. The sample pieces were then shaken for 1 h in soapy water, put in perforated stainless steel containers ("tea balls," 40 mm diam.), and washed overnight in a pipette washer with a flow rate of 1 $L \cdot min^{-1}$ of water. The samples were dried by hanging the containers for 24 h in a laminar airflow cabinet, then sterilized in 0.5% NaOCl for 2–3 min, followed by three rinses in sterile distilled water. The samples were air dried on sterile filter paper. Crown and subcrown internodes were separated and both were divided: the crown was split lengthwise and the subcrown was split into a top part (adjacent to crown) and a base part (adjacent to seed), to be plated on two different isolation media.

The PD^+ medium was based on the modified potato dextrose agar (Burgess et al. 1988), which consisted of half-strength potato dextrose agar (19 $g \cdot L^{-1}$ Difco PDA and 7.5 $g \cdot L^{-1}$ Difco Agar), 50 $mg \cdot L^{-1}$ kanamycin sulfate (added before autoclaving), 100 $mg \cdot L^{-1}$ streptomycin sulfate, and 7 $mg \cdot L^{-1}$ DCNA (98% 2,6-dichloro-4-nitroaniline, Aldrich Chemical Company, U.S.A.). Both streptomycin and DCNA were added in sterile water after autoclaving and cooling the medium to 55°C. PD^+ is a general isolation medium whereby most fungi show restricted colony growth compared with full-strength PDA. *Cochliobolus sativus* formed clear, distinct colonies rarely exceeding 2 cm diam. after 10 d (Burgess et al. 1988).

Table 1. Comparions of average subcrown-internode score (SIS), average percent diseased plants (PDP), and percent isolation of *Cochliobolus sativus* and *Microdochium nivale* from each agricultural stability zone surveyed in Syria in 1992 and 1993.

				% isolation			
	No. of	Average		*C. sativus*		*M. nivale*	
Zone	fields	SIS	PDP	Mean	Range	Mean	Range
1992 survey							
1	12*	3.0 b	66 a	32 bc	0–89	36 a	0–67
2	24	3.1 b	66 a	20 c	0–78	41 a	5–97
3	12	3.8 a	80 a	51 ab	0–100	21 a	0–74
4	5	3.6 ab	77 a	66 a	20–100	26 a	0–90
1993 survey							
1	5†	1.5 b	22 a	44 ab	2–82	35 a	8–80
2	41	2.8 a	53 b	28 b	0–100	56 b	4–100
3	14	3.2 a	64 b	62 a	0–100	35 a	0–98
4	12	3.1 a	62 b	46 ab	7–98	46 a	0–93

Note: Means within the same column and year that are followed by the same letter are not significantly different according to a protected LSD test ($P < 0.05$).

*Of the 12 barley fields samples in zone 1 during 1992, 7 were irrigated and 5 were located in the high rainfall Ghab valley in west Syria.

†Of the 5 barley fields sampled in zone 1 during 1993, 2 were irrigated and 3 were located in the high rainfall Jinderess area in west Syria.

The CD$^+$ medium consisted of Czapex–Dox agar with one-quarter of the normal saccharose (7.5 g·L^{-1} saccharose), amended with 50 mg·L^{-1} kanamycin sulfate (added before autoclaving), 100 mg·L^{-1} streptomycin sulfate (added as with PD$^+$ described above) and 5 mg·L^{-1} benomyl (50% Benlate wettable powder from Dupont, added to media in 1 mL 95% ethanol after autoclaving and cooling). This medium is selective against *Fusarium* spp. (Stack 1977).

Plates were incubated for 2 d in the dark at 20°C and then examined. After another 3 d incubation at 18°C with 12 h light, plates were observed for new fungal growth every 2 d for 1 week. Identification of the fungi was based on colony colour, appearance, growth rate, and microscopic examination of spores. Red-pigmented *Fusaria* were identified by Professor L.W. Burgess of the University of Sydney, Australia. Identification of *M. nivale* and *Microdochium bolleyi* (Sprague) de Hoog & Hermanides-Nijhof was confirmed by the Central Bureau for Fungal Cultures in Baarn, Netherlands.

Pathogenicity tests

Fungi were tested for pathogenicity using seedlings grown under semisterile conditions. Glass culture tubes (20 cm long, 2.5 cm diam.) with 20 mL Hoagland nutrient solution No. 2 (Hoagland and Arnon 1950) and 6 g·L^{-1} agar were closed with a cotton plug and autoclaved at 120°C for 1 h. Barley seeds of the susceptible, but widely grown cultivar 'Arta' were surface sterilized in 1% NaOCl for 3 min, rinsed twice in sterile distilled water, and germinated for 2 d on wet sterile filter paper in Petri dishes at 18–20°C in the dark. Two germinated seeds were placed in each tube and grown at 18–20°C and 16 h light. Contaminated tubes were discarded after 4 d, when the first leaf started to unfold. A 7- to 10-day-old mycelium plug (0.5 cm diam.) of the fungal isolate was placed on the medium next to the seedling. Three weeks after inoculation, plants were scored for discolouration using a 0–5 scale, keeping the value of the ratings in similar proportion to the SIS: 0 = clean, 0.5 = slight discolouration of the coleoptile, 1 = coleoptile completely brown, 1.5 = light browning of stem base, 2 = dark brown lesion of at least 1 cm long at the stem base, 2.5 = stem lesion extending until first leaf, 3 = necrosis of parts of first leaf, 3.5 = first leaf dead, 4 = necrosis of parts of second leaf, 4.5 = less than 10% of plant still green, 5.0 = plant completely dead. Isolates that produced disease ratings of 2 or more were considered pathogenic. Stem bases were harvested after scoring, washed in tap water, dried, and stored in small paper bags at 5°C. Fungi were re-isolated after surface sterilization in 0.5% NaOCl for 1 min. Isolates similar to the original cultures were generally re-isolated from plants scoring 0.5 or higher.

Statistical analyses

Data on disease ratings and isolation frequencies among agricultural stability zones and geographical regions were analyzed using ANOVA (Genstat 5 Release 3.2, PC/Windows 95, © 1995, Lawes Agricultural Trust, Rothamsted Experimental Station). Location effects were separated using LSD tests at $P \leq 0.05$. Pearson's correlation coefficient and Student's paired *t*-test were used to relate the incidence of *C. sativus* and *M. nivale* within fields. Pearson's correlation coefficient was also used to correlate average disease ratings with other notes taken in the field. The efficiency of both media for isolation of *C. sativus*, and the relationship between SIS scoring categories and isolation frequencies of different fungi, were analyzed with Chi-square tests. The relationship between SIS and tiller number was studied by grouping plants in four categories (SIS = 2, 1 tiller; SIS = 2, >1 tiller; SIS ≥ 3, 1 tiller; SIS ≥ 3, >1 tiller) and analyzing the frequencies of the different categories by a Chi-square test.

Results

Root rot symptoms

Agricultural stability zones differed for average SIS per field ($P < 0.05$), but not PDP in 1992. In 1993, differences among zones were significant for both average SIS ($P < 0.01$) and PDP ($P < 0.05$). There was more severe root rot in the drier zones (Table 1). When fields in zones 1 and 2 were combined and tested by ANOVA against the combined fields of zones 3 and 4, lower SIS and PDP values were found in the higher rainfall areas, both in 1992 and 1993 ($P < 0.05$, data not shown).

Other root disorders

Nematode cysts were found on barley roots in 16 fields during the 1992 survey (13/31 fields in the west geographical region and 3/7 fields in the northwest region) and in 18 fields of the 1993 survey (10/22 fields in the west region and 8/13 fields in the northwest region). No cysts were

Table 2. Relationship of healthy and diseased classes (based on subcrown-internode scores, SIS) with number of tillers per plant in four agricultural stability zones surveyed in Syria in 1992 and 1993.

		Healthy (SIS = 0–2)			Diseased (SIS = 3–5)			
Zone	No. of plants	% plants with 1 tiller	% plants with >1 tiller	Avg. no. of tillers	% plants with 1 tiller	% plants with >1 tiller	Avg. no. of tillers	Probability of χ^2 test
1992 survey								
1	261	11.5	17.6	2.1	44.1	26.8	1.7	0.001
2	455	18.2	15.2	1.7	38.5	28.1	1.7	>0.100
3	237	13.1	7.2	1.6	48.5	31.2	1.7	>0.100
4	98	7.1	16.3	2.4	23.5	53.1	2.4	>0.100
All	1051	14.4	14.1	1.8	40.7	30.8	1.8	0.059
1993 survey								
1	249	27.7	50.2	2.4	10.9	11.2	1.9	0.069
2	2027	25.9	21.1	1.8	35.3	17.7	1.4	0.001
3	696	20.3	15.7	1.6	42.9	21.1	1.5	0.005
4	599	24.4	14.2	1.6	45.4	16.0	1.4	0.006
All	3571	24.7	20.9	1.8	36.8	17.6	1.4	0.001

found east of the Euphrates river (north and northeast regions).

Heavy infestations (>90% of the plants affected) of the roots with red nodules known locally as ground pearl (*Porphyrophora tritici* Bod.) were found in 3 fields (1 in the west and 2 in the north regions) in 1992 and 11 fields (6 in the west, 1 in the northwest, 3 in the north, and 1 in the northeast regions) in 1993. As far as cropping history could be traced, ground pearl infestations were restricted to fields that had barley in the previous year. In one irrigated durum wheat field, a heavy infestation was found on volunteer barley plants, while the wheat was not affected.

Take-all [*Gaeumannnomyces graminis* (Sacc.) Arx. & Olivier var. *tritici* (Walker)] was only found in one excessively irrigated wheat field in the western region. The identity of the pathogen was confirmed by plating on selective media (Juhnke et al. 1984). Up until this time, *G. graminis* had not been reported in Syria (Mamluk et al. 1992).

Relationship of SIS and number of tillers

In 1992, a Chi-square test showed that plants with single tillers also had higher SIS than plants with more tillers in zone 1 (Table 2). This relationship was not significant in the other zones, although the total number of plants over all zones showed a negative trend between tiller number per plant and SIS. In 1993, plants with single tillers had higher SIS in zones 2, 3, and 4 as well as the total number of plants over all zones. When fields were analyzed separately, regardless of the agricultural stability zone, only a few fields showed significant differences (2/53 fields in 1992 and 5/73 in 1993).

Isolated fungi and efficiency of media

In 1992, isolations from a total of 1020 plants yielded 31% *C. sativus* and 35% *M. nivale*. In 1993, isolations from 1120 plants (including both samples from the 12 fields that were sampled twice) yielded 38% *C. sativus* and 51% *M. nivale*. Red-pigmented *Fusaria* were isolated from 12% of the plants in 1992 and from 13% in 1993. *Microdochium bolleyi* was isolated from 8% of the plants in 1992 and from 7% in 1993.

Based on Chi-square tests of all samples collected in 1992 and 1993, the isolation frequency of *C. sativus* with CD^+ medium was more efficient in 1992 (PD^+ 27%, CD^+ 31%, $P < 0.001$) and 1993 (PD^+ 30%, CD^+ 38%, $P < 0.001$). Therefore, isolation frequency of *C. sativus* was based on counts from the CD^+. Counts of other fungi were based on their growth on PD^+.

Dividing both the crown and the subcrown internode of each plant for plating on the two media (one general and one selective for *C. sativus*) was expected to help in detecting mixed infections of *C. sativus* and other prevalent fungi, such as *M. nivale*. Fewer than 7% of all plants had mixed infections. The improvement in detection of mixed infection using the two media as compared with using only PD^+ was not significant in either year. There was no significant difference in the isolation of *C. sativus* and *M. nivale* from either the top or base portions of the subcrown internode.

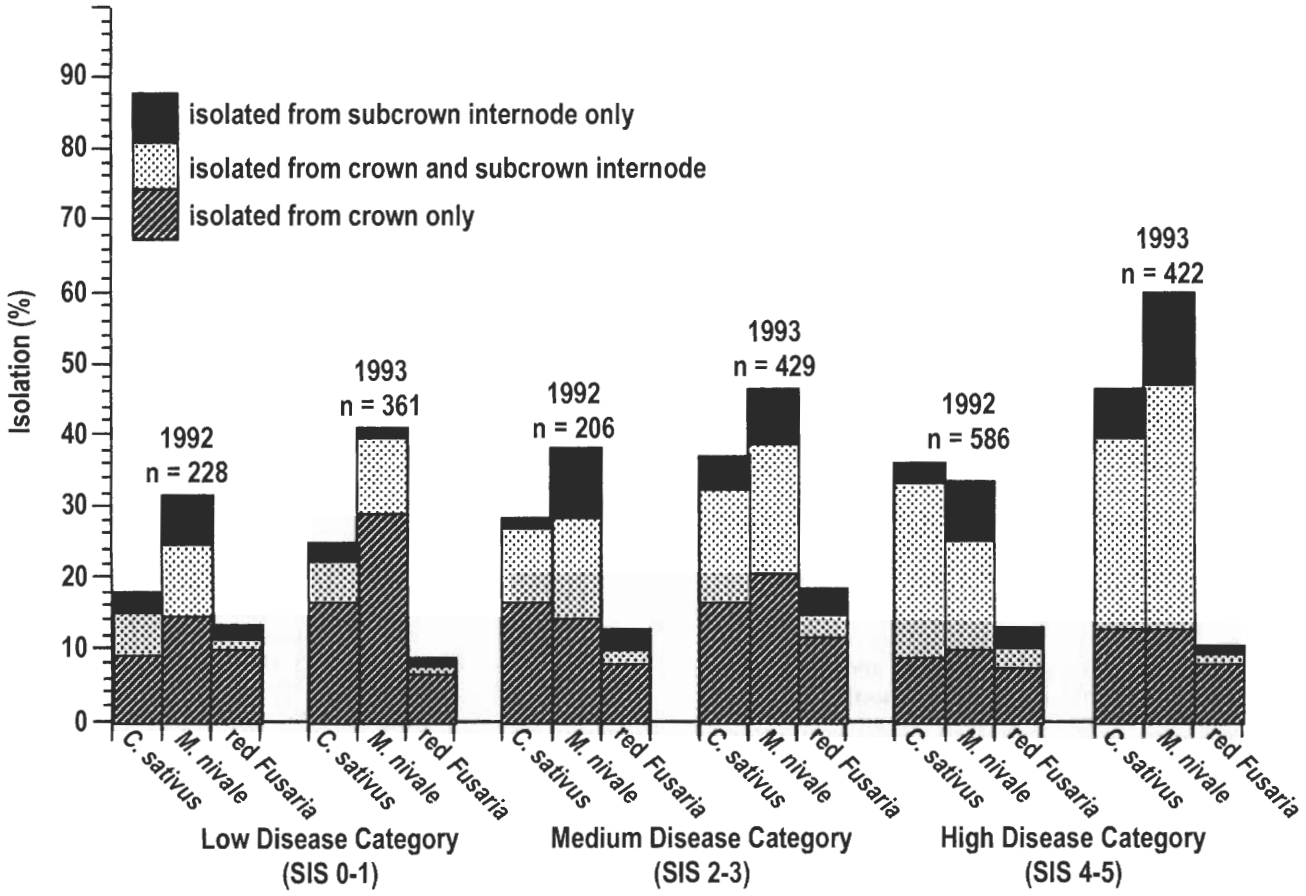
Relationship between isolation frequency and SIS

The isolation frequencies of *C. sativus*, *M. nivale*, and red *Fusaria* from crowns and subcrown internodes of plants rated in the low (SIS 0–1), medium (SIS 2–3), and high (SIS 4–5) disease categories are shown in Fig. 2. In both years, the isolation frequency of *C. sativus* differed between the three categories, with higher isolation occurring with higher SIS (Chi-square test, $P < 0.001$). A similar relationship was observed for *M. nivale* in 1993 (Chi-square test, $P < 0.001$). In the low disease category, both *C. sativus* and *M. nivale* were mostly recovered from crown tissue, but in the higher SIS categories these fungi were recovered from both the crown and subcrown internode (SCI). There was no relationship between SIS and the isolation of red-pigmented *Fusaria*, which were mainly recovered from crown tissue.

Differences in pathogen isolations among and within agricultural stability zones

The mean isolation of *C. sativus* was significantly different among the four agricultural stability zones surveyed in 1992 and 1993 (Table 1). The mean isolation of *M. nivale*

Fig. 2. Isolation of *Cochliobolus sativus*, *Microdochium nivale*, and red-pigmented *Fusaria* from root tissues (crowns and subcrown internodes) of plants (*n*) with subcrown-internodes scores (SIS) in the low (0–1), medium (2–3), and high (4–5) disease categories collected in Syria in 1992 and 1993.



did not differ among the zones. There was a large variation in range of isolation observed for both pathogens.

A comparison was made between zones 2 and 3, which had a relatively large number of fields surveyed in both years. ANOVA showed that the isolation of *C. sativus* was higher in zone 3 ($P < 0.01$), while the isolation of *M. nivale* was higher in zone 2 ($P < 0.05$).

There was a negative correlation in the isolation frequency of *C. sativus* and *M. nivale* in both years (1992, $r = -0.53$ at $P < 0.001$ and 1993, $r = -0.60$ at $P < 0.001$). However, in some fields that had a high (>30%) isolation frequency of both pathogens, the number of plants infected with both *C. sativus* and *M. nivale* was similar to the number of plants where only a single pathogen was isolated. Therefore, there was no indication that infection by one pathogen excluded infection by the other one.

Differences in pathogen isolations among geographical regions

The larger number of fields sampled within zone 2 made it possible to compare geographical regions (Table 3). The SIS and PDP were not significantly different among the west, northwest, north, and northeast regions in 1992. In 1993, both the SIS and PDP were higher in the north and northwest (ANOVA at $P < 0.001$). The greatest number of isolations of *M. nivale* was from the northwest region, whereas this region had the fewest isolations of *C. sativus*.

Comparison of early versus late sampling

Root rot scores (SIS and PDP) significantly increased within the 12 fields sampled twice in the northeast region between late April and late May 1993. Using a Student's paired *t*-test ($P < 0.001$), the average SIS and PDP were higher at maturity (SIS = 3.0, PDP = 83) than at flowering (SIS = 2.6, PDP = 51). However, the pattern of isolation of *C. sativus*, *M. nivale*, and red-pigmented *Fusaria* did not change from flowering to maturity.

Pathogenicity tests

Pathogenicity tests were conducted for 252 isolates from the 1992 survey and for 738 isolates from the 1993 survey representing an array of different fungi. Over 80% of the *C. sativus* and *M. nivale* isolates, but less than 20% of the *Fusaria* and the *M. bolleyi* isolates, were pathogenic (Table 4). Only 4 of the 11 representative *Fusaria* cultures sent for identification were keyed to species, because the remaining cultures failed to sporulate after re-isolation. *Fusarium acuminatum* Ell. & Ev. and *Fusarium equiseti* (Cda.) Sacc. were identified. Both of these species are generally regarded as saprophytes (Burgess et al. 1988), but one isolate of *F. acuminatum* was highly pathogenic (rated

Table 3. Comparisons of the subcrown-internode score (SIS), percent diseased plants (PDP), and percent isolation of *Cochliobolus sativus* and *Microdochium nivale* in four geographical regions located in agricultural stability zone 2 in Syria in 1992 and 1993.

Region	No. of fields	SIS		PDP		*C. sativus*		*M. nivale*	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
1992 survey									
West	10	3.0 a	1.4–4.2	65 a	10–100	29 a	5–78	42 ab	5–76
Northwest	7	3.2 a	2.3–3.5	69 a	57–80	6 a	0–11	60 b	15–95
North	5	3.4 a	2.8–4.2	72 a	63–94	20 a	0–43	14 a	5–25
Northeast	2	2.1 a	1.6–2.6	47 a	33–60	24 a	15–32	39 ab	30–47
1993 survey									
West	11	1.8 a	1.2–2.4	27 a	12–42	34 b	0–100	44 a	4–92
Northwest	13	3.4 c	2.3–4.5	64 c	32–90	13 a	0–30	72 b	25–100
North	5	4.5 d	3.9–4.8	96 d	88–100	30 ab	0–98	55 ab	19–92
Northeast	12	2.4 b	1.6–3.2	46 b	21–68	36 b	4–67	52 ab	6–100

Note: Means within the same column and year that are followed by the same letter are not significantly different according to a protected LSD test ($P < 0.05$).

Table 4. Distribution of fungal isolates by pathogenicity classes in 1992 and 1993.

Fungus	1992 survey				1993 survey			
	% per class*			No. of isolates	% per class*			No. of isolates
	<2	2–4	>4		<2	2–4	>4	
C. sativus	8	63	29	48	21	54	25	136
M. nivale	12	86	2	42	19	54	27	250
Red-pigmented *Fusaria*	71	20	9	45	80	18	1	92
Other *Fusaria*	77	22	1	90	85	11	4	181
M. bolleyi	92	8	0	13	80	20	0	40
Others	93	7	0	14	87	13	0	39

*Pathogenicity was rated on a 0–5 scale, where values greater than 2 indicate that the fungus is pathogenic.

5 on the pathogencity scale) in the seedling tests; the other three isolates were not pathogenic.

Discussion

In this study, the major root pathogens of barley were *C. sativus* and *M. nivale*, with more than 80% of the isolates demonstrating moderate to high pathogenicity. *Microdochium bolleyi* and some *Fusarium* spp. were minor pathogens of barley, with less than 20% of the isolates demonstrating moderate pathogenicity. Mamluk et al. (1992) did not report finding *M. nivale* and *M. bolleyi* as pathogens in Syria; the current study is the first report. *Fusarium* spp. were only isolated from 12–13% of the plants in both years, and most isolates were not pathogenic. There was only one confirmed case of take-all on wheat under irrigation, which suggests that this disease is not important in this area at this time. White nematode cysts on barley were only found in the north and west geographical zones, while ground pearl was restricted to fields with continuous barley cultivation or barley volunteers.

The severity of root diseases was higher in drier areas (agricultural stability zones 3 and 4) than in the wetter regions (agricultural stability zones 1 and 2) in both years. On average, *C. sativus* was recovered more frequently in zones 3 and 4 and less frequently in zones 1 and 2. *Microdochium nivale* was recovered more frequently in zone 2. These observations suggest that *C. sativus* occurred to a greater extent in the drier areas, whereas *M. nivale* occurred more frequently in the moister areas.

Microdochium nivale was also recovered more frequently than *C.sativus* in the northwest region (Al Bab province). Farmers in this area have practised continuous barley cultivation since the early 1970s, while the trend to continuous cereal cultivation from barley–fallow systems is more recent in other regions of Syria. Sturz and Bernier (1989) reported an increase of *M. nivale* under continuous wheat cultivation in Canada. Results from fungal isolations in long-term rotation trials in northern Syria showed increased levels of *M. nivale*, but not of *C. sativus*, under continuous barley (van Leur 1993). Therefore, changing patterns in cultivation may also influence the prevalence of this disease.

A comparison of the disease intensity and pathogen population at flowering and maturity showed that the pattern of fungal isolation did not change, but the SIS and PDP increased over time. As the plants matured, there was increased stress from heat and drought. Duczek (1993) found that common root rot severity increased with higher concentration of soil salinity, but the number of colonies of *C. sativus* per gram of soil did not change. The severity of common root rot increases with conditions contributing to higher plant stress, such as drought, flooding, or salinity

(Bailey et al. 1989; Duczek 1986, 1993). Stress creates a change in symptom expression that does not necessarily result from increases in the pathogen population.

Yield losses from common root rot have been related to a reduction in plant emergence (Grey et al. 1991) and fertile tillers (Duczek 1989; Wildermuth et al. 1992). When calculated over all of the agricultural stability zones, there was a weak negative correlation between disease severity and number of tillers per plant. Similarly, Tinline and Ledingham (1979) showed that disease intensities and yield losses were highly correlated in wheat but in barley the association was more variable. Barley cultivars with intermediate disease reactions had the lowest yield loss. They concluded that barley and wheat have differences in tolerance to common root rot. Bailey and Wolfe (1994) found that the progeny of a cross between a resistant barley cultivar and a tolerant barley cultivar had weak negative correlations between disease severity and yield. However, this association became stronger when the tolerant progeny were removed from the regression, leaving only the resistant and susceptible progeny. Barley fields in Syria are highly heterogeneous in many traits and likely comprise a mixture of plants with resistant, susceptible, and tolerant genotypes. Since tolerant genotypes have the ability to yield well under high disease pressure, these genotypes may have weakened the correlation between disease severity, number of tillers, and the ultimate contribution to yield. Using soil fumigation techniques, disease losses from root rot complexes in barley may go as high as 30% (Bailey et al. 1997). In the same study a comparison was made using seed inoculation techniques with only *C. sativus*, and yield losses were about 11% in barley. Either yield losses due to common root rot were being underestimated or more than one fungus was responsible for yield reductions in barley. In Syria, the survey showed that a fungal complex caused root disease. A controlled study is needed to quantify the relationship between the root rot complex, agronomic characters, and environmental effects.

The techniques used in this study favored *C. sativus* over *M. nivale* because *C. sativus* could be isolated on both media, while *M. nivale* could only be isolated from the PD^+ medium. The optimal temperature for growth of *C. sativus* is 20–25°C (Wiese 1987), whereas for *M. nivale*, the optimal growth occurs at below 15°C (Cassini 1981). Similarly, van Leur (1993) found that an incubation temperature of 8°C doubled the isolation frequency of *M. nivale* compared with 20°C. The incubation temperature of 20°C used in the present study favored growth of *C. sativus* and probably limited the growth *M. nivale*. Using only selective media such as CD^+ to determine causal agents of root rot should be avoided.

Handling of crown and SCI pieces separately during the isolation process showed that isolation frequencies of *C. sativus* and, to a lesser extent, *M. nivale* on both plant parts were positively related to the discolouration of the SCI. This supports the conclusion of Stack (1986) that rating SCIs for common root rot symptoms also gives an indication of the level of infection of other belowground plant parts. The selection of plants with greater than 1 cm facilitated disease rating and did not affect pathogen isolation. Crown tissue was more often heavily infested by *C. sativus* and *M. nivale* than the SCI. Tinline et al. (1994) showed that crown tissue was infested earlier than SCIs by *C. sativus*. Since there was a strong association between infestation on crowns and SCI, future isolation work need only recover from crown tissue.

In conclusion, *C. sativus* and *M. nivale* were the most prevalent and highly pathogenic fungi in the barley production areas of northern Syria. Isolation of the pathogens was associated with increasing disease severity on barley plants. *Cochliobolus sativus* was more common in drier areas, whereas *M. nivale* was more prevalent in the wetter areas and the northwest region.

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