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IMPACT OF DIMETIPHINE, ETEPHON AND DIQUAT ON THE HEALTH STATUS OF WHITE LUPIN SEEDS (*Lupinus albus* L.)

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ABSTRACT

The research investigated the seeds of 'Bardo' traditional cultivar, and 'R-141' self-completing white lupin cultivar. Over 1996-1998, once the seeds reached their physiological maturity, the plants were treated with Harvade 250 SC (dimetiphine), Ethrel (etephon), plant growth regulators and Reglone (diquat), a desiccant. Seeds harvested from plants whose sowing material was vernalised and two-stage harvest seeds constituted objects 4 and 5. The seeds collected each year were divided into two categories based on a visual *'healthy and infected'* selection. The share of the latter group in the seed yield of both two-stage harvested cultivars was highest, while having applied Ethrel ('Bardo) and Harvade '(R-141') as well as following seed vernalisation (both cultivars), the share turned out lower. 'R-141' self-completing yield included significantly fewer seeds with fungal infection symptoms than the 'Bardo' traditional cultivar. *'Healthy and infested'* seeds of each combination were placed on the PDA medium. A total number of 18 fungal species were identified. *Colletotrichum gloeosporioides, Alternaria alternata and Cladosporium herbarum* constituted the dominating group on infected seeds and *Cladosporium herbarum* on healthy ones. Dimetiphine and diquat limited the number of dominant species to *C. herbarum* and *C. gloeosporioides*, while the two-stage harvest increased the number of dominant species by two, *B. cinerea* and *A. alternata*, especially in 'R-141'.

Key words: desiccants, dimetiphine, diquat, etephon, growth regulators, seed health status, white lupin

INTRODUCTION

Under average weather conditions white lupin gives 3-4 tonne seed yield. White lupin pods neither shatter nor fall off, which makes it possible to start harvesting when the seeds have desiccated naturally in the field [13]. However over higher-rainfall years, white lupin ripens as late as in September when pods and seeds often contain too much water to be single-stage harvested. White lupin seeds are physiologically mature as soon as they contain 50-60% of water [15]. Seed desiccation in pods on the mother plant (two-stage harvest) or even in pods under laboratory conditions does not affect biological seed value and vigour, yet seed harvest at this maturity stage is not feasible due to excessive stem and pod juiciness. The period from seed physiological to full morphological maturity allowing for a single-stage harvest can coincide with seed deterioration on the mother plant, which is observed especially over delayed ripening when pods and seeds are exposed to fungal pathogens; hence low sowing and fodder values. The most frequent pathogenic fungi occurring on lupin seeds include *Colletotrichum gloeosporioides* and various species of *Fusarium* genus [6,9,18,19,23]. Even over years favourable to ripening, white lupin seed yield contains from a few to several percent of deformed, wrinkled and decolourised, to various extent, seeds as well as seeds with visible fungal spots [20].

White lupin generative period lasts, depending on the cultivar, from about 100 to 110 days. Flowering and vegetation periods of new self-completing cultivars are shorter by a few to several days, as compared with traditional cultivars [22]. Additionally traditional form plants branch out heavily accounting for about 50% of the seed yield (Phot. 1), unlike self-completing forms whose main stem develops 100% of the seed yield. [20,22]. The research hypothesis assumes that the application of substances different in their activity and speed of plant tissue desiccation can affect the seed health status as well as the composition of microorganisms occurring on seeds.

The present research aims at defining the impact of selected growth regulators, desiccants, artificial seed vernalisation and two-stage harvest on the yield health status as well as on the composition of fungal microflora occurring on traditional and self-completing white lupin cultivar seeds.

Phot. 1. 'Bardo' and 'R-141' white lupin plants over generative development

MATERIAL AND METHODS

The research investigated strict-field-experiment white lupin seeds harvested over 1996-1998; the field experiment defined the impact of 2 $l \cdot ha^{-1}$ Harvade 250 SC (dimetiphine) and Ethrel (etephon), growth regulators, as well as 3 $l \cdot ha^{-1}$ Reglone (diquat), a plant desiccant, on 'Bardo' traditional and 'R-141' self-completing white lupin cultivar seed yield and its health status. Seeds harvested from plants obtained from 5-6^{o}C 14-day conditioned and vernalised seeds, from plants threshed following a two-stage harvest as well as from non-treated plants constituted the other experimental objects. A two-stage harvest and plant spraying took place 4-7 days following physiological seed ripening when seeds of 'Bardo' contained on average 50.9% and of 'R-141' 45.1% of water. Over successive research years 'Bardo' and 'R-141' plants were sprayed on September 6 and 3, September 20 and 15 and on August 27 and 17, respectively. 'Bardo' reached the full morphological maturity after 152, 150 and 148 days and 'R-141' after 145, 142 and 143 days.

Four-replication field experiment on 18 m^2 plots was carried out compliant with white lupin agronomic practices. Cross-cultivar sowing rate per 1 m^2 differed; 75 germinating seeds for traditional cultivar and 100 seeds for self-completing cultivar.

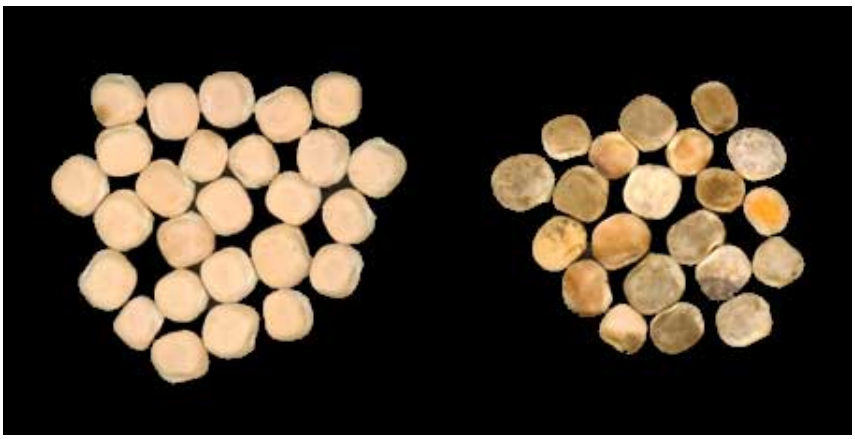
The seeds collected each year were divided into two categories based on a visual *'healthy and infected'* selection, without decolourised seed coat and whose seed coat colour showed fungal infection symptoms, respectively
(Phot. 2).

Phot. 2. 'Bardo' and 'R-141' white lupin *'healthy and infected'* seeds

'Bardo'

Healthy seeds **Infected seeds**

'R-141'



100 of *'healthy and infected'* seeds of each cultivar and combination were mycologically investigated. The seeds were washed under running water over 30 minutes and then three times in sterile distilled water and placed, in groups of 6, on Petri dishes filled with the PDA medium, acidified with citric acid up to pH 5.5. After 10 days of incubation at 24°C fungal colonies were identified following mycological guidelines [1,2,4,5,8,11,17].

RESULTS AND DISCUSSION

The seed ripening period length as well as water and temperature conditions are essential for lupin yielding and quality of seeds harvested [13,16,20]. Over 1996 -1998 lupin ripening rainfall exceeded 200 mm, while in 1997 it amounted to about 150 mm (Table 1). In 1997 average air temperature was highest, which enhanced pod and seed desiccation and could eliminate plant desiccation treatment. The self-completing cultivar generative and vegetation periods, similarly to the reports by Stawiński [20] and Święcicki [22], were shorter than the respective periods of the 'Bardo' traditional cultivar.

Table 1. Weather conditions from-physiological-to-full white lupin seed maturity

Physiological-to-full seed maturity period characteristics	Bardo			R-141		
	1996	1997	1998	1996	1997	1998
Period, days	97	81	88	96	78	85
Mean air temperature, °C	15.0	18.2	16.7	15.2	18.0	16.3
Total rainfall, mm	252	157.4	229	254.2	156.2	216

The cultivars researched, despite a few-day variation in the vegetation period length [16] were harvested on the same day. The self-completing 'R-141' seed yield contained significantly fewer seeds with visible fungal symptoms, especially following the application of Harvade 250 SC and in the control yield, as compared with 'Bardo' (Table 2), which must have been due to a varied share of seeds developed on the main stem and lateral branches in the total seed yield. 100% of the seed yield of self-completing cultivars comes from the main non-branching stem and the pods and seeds ripen simultaneously [20,22]; hence their higher transpiration and desiccation from the very beginning. As for the traditional form, about 50% of the seed yield originates from the main stem and about 50% from lateral branches. The pod and seed development and ripening on lateral branches are recorded a little later and not so simultaneous, as compared with the seeds on the main stem, and generally under conditions more favourable to fungal growth [16].

Table 2. Share (%) of fungal disease-symptom seeds in the white lupin seed yield

Treatment	Bardo	R-141
Harvade 250 SC	15.9 Aab	10.6 Bb
Reglone	14.8 Aab	11.6 Aab
Ethrel	13.4 Aab	10.4 Ab
Seed vernalisation	12.7 Ab	10.7 Aab
Two-stage harvest	17.9 Aa	15.2 Aa
Control	16.2 Aab	10.9 Bab
Mean	**15.1 A**	**11.6 B**

Other favourable-to-seed-health-status treatments, especially for 'Bardo', included sowing material vernalisation and, for 'R-141 – etephon application. Łączyńska-Hulewiczowa [12] also observed a favourable impact of seed vernalisation on white lupin seeds and plants health status obtained from those seeds.

The two-stage harvest of both cultivars deteriorated the seed health status considerably. Generally it is believed that seed ripening in pods following two-stage harvest enhances the seed viability and vigour and such seeds are often healthier than single-stage harvested seeds [13,15]. Although white lupin plant mowing over physiological seed maturity suddenly interrupted plant vegetation, a higher, as compared with the single-stage harvested seeds, two-stage-harvested seed infection could have been due to a slow seed desiccation interchangeably with moistening when accompanied by high rainfall, especially over 1996 and 1998.

The mycological analysis showed that the control healthy lupin seeds were infected mostly by *Cladosporium herbarum* as well as *Alternaria* and *Penicillium* - saprophytic fungi - and by *Botrytis cinerea* and *Fusarium* spp. - pathogenic fungi (Table 3). Seeds with visible fungal infection showed also high intensity of *Colletotrichum*

gloeosporioides infection (Phot. 3) – the pathogenic fungus unobserved on the healthy seeds. Over 1996 no *Colletotrichum gloeosporioides*, while in 1998 as many as 4-6 isolates of that genus - on '*healthy seeds*' and as many as 70-79 on '*infected seeds*' (Table 4) were isolated. A sudden occurrence of anthracnose caused by *C. gloeosporioides* has been observed since early 90s. The anthracnose epidemic of recent years, both in Poland and the neighbouring countries, has been responsible for a slump in the area of more susceptible yellow and white lupin species plantation [7,10].

Table 3. Total number of pathogenic and saprophytic fungal isolates on control

Species	Bardo				R-141			
	1996	1997	1998	Total	1996	1997	1998	Total
Alternaria alternata (Fries.) Keiss.	12	8	16	36	14	12	16	42
Alternaria spp.	2	-	-	2	2	-	-	2
Aspergillus niger van Tieghen	2	3	-	5	3	2	-	5
Botrytis cinerea Pers.	-	17	-	17	4	26	2	32
Candida albicans (Robin) Berk.	2	4	-	6	2	7	-	9
Cladosporium herbarum (Pers.) Link. ex Fr.	28	42	40	110	38	28	36	102
Colletotrichum gloeospo-rioides Penz.	-	2	6	8	-	4	4	8
Fusarium avenaceum (Fr.) Sacc.	-	-	3	3	-	8	1	9
Fusarium culmorum (W.G. Smith) Sacc.	2	-	-	2	-	4	-	4
Fusarium solani (Mart.) Sacc.	-	3	4	7	-	2	-	2
Penicillium spp.	4	6	-	10	8	36	4	48
Phoma sp.	2	-	-	2	-	-	-	-
Total	**54**	**85**	**69**	**208**	**71**	**129**	**63**	**263**

Phot. 3. Aecervulus of *Colletotrichum gloeosporioides* Penz.

Table 4. Total number of pathogenic and saprophytic fungal isolates on control white lupin *'infected seeds'*

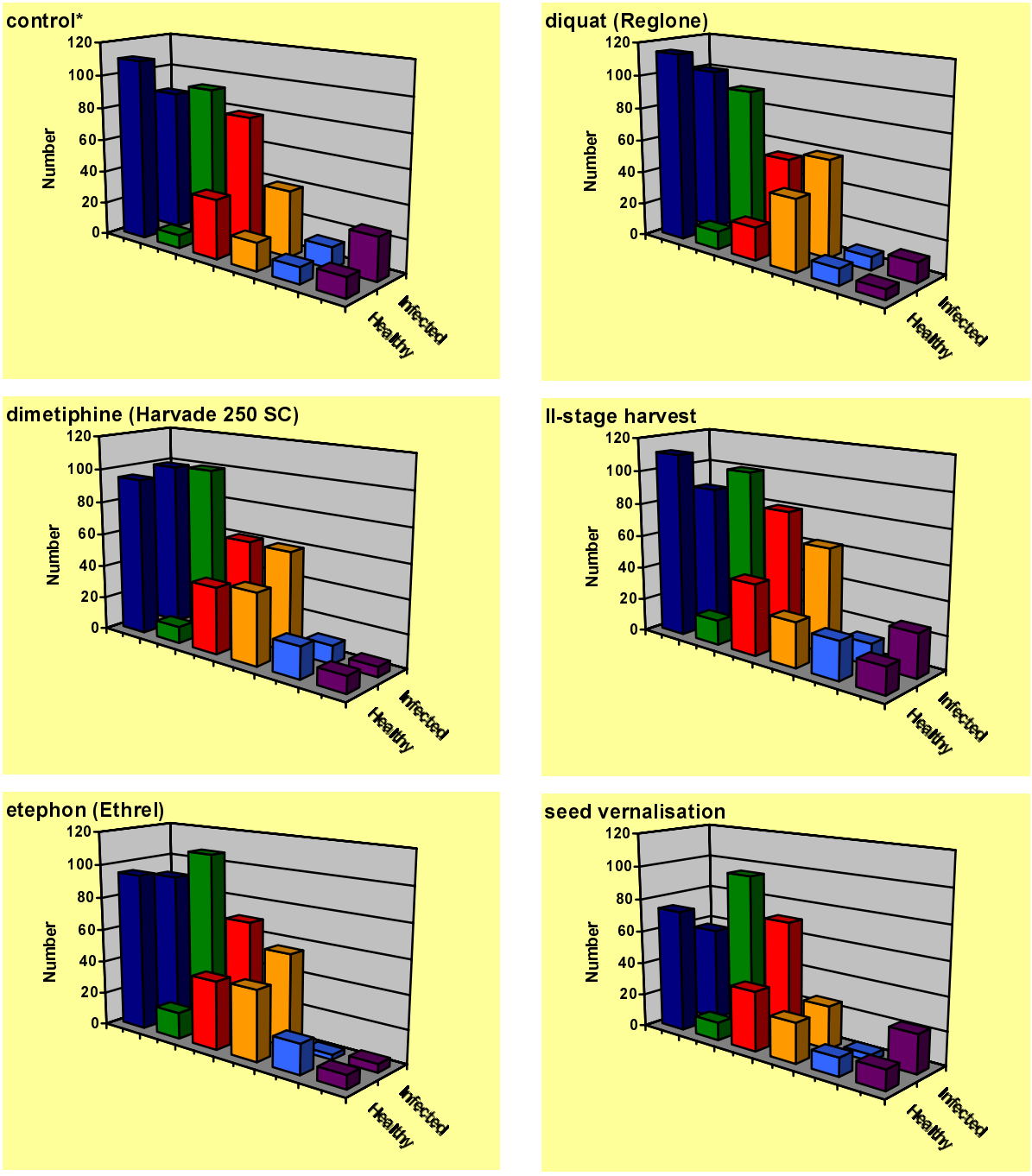
Species	Bardo				R-141			
	1996	1997	1998	Total	1996	1997	1998	Total
Alternaria alternata (Fries.) Keiss.	23	28	28	79	29	34	28	101
Alternaria spp.	-	-	-	-	4	-	-	4
Aspergillus niger van Tieghen	12	-	-	12	-	7	-	7
Botrytis cinerea Pers.	16	24	-	40	7	28	2	37
Candida albicans (Robin) Berk.	6	4	-	10	10	6	-	16
Cladosporium herbarum (Pers.) Link. ex Fr.	34	46	6	86	42	36	12	90
Colletotrichum gloeospo-rioides Penz.	-	22	70	92	-	14	79	93
Fusarium avenaceum (Fr.) Sacc.	6	3	2	11	2	6	-	8
Fusarium culmorum (W.G. Smith) Sacc.	5	-	2	7	2	4	2	8
Fusarium solani (Mart.) Sacc.	-	4	4	8	-	2	-	2
Penicillium spp.	7	4	2	13	6	8	-	14
Phoma sp.	-	2	-	2	2	2	-	4
Total	**109**	**137**	**114**	**360**	**104**	**141**	**123**	**384**

The control healthy seeds and seeds with visible fungal symptoms showed a similar number of saprobiotic fungi and their species composition. The pathogenic fungi, however, were isolated on '*infected*' seeds more frequently, as compared with the '*healthy*' seeds. *Colletotrichum gloeosporioides* was isolated on as many as 185 seeds with visible fungal infection symptoms and on 16 '*healthy*' seeds (out of 600 analysed), while *Botrytis cinerea* - on 77 and 49, respectively. *Fusarium* was isolated almost twice as often on '*infected*' than on '*healthy*' seeds. Despite a slightly higher health status of the self-completing cultivar the number of isolates and the saprobiotic and pathogenic species composition observed on seeds of both cultivars were similar.

The preparations applied differed considerably in their activity. Diquat, an active substance of Reglone, destroys cell membranes [21], dimetiphine contained in Harvade 250 SC prevents stoma from closing up and enhances leaf and pod transpiration [14]. 2-chloroethanephosphonic acid, Ethrel active substance, stimulates protein decomposition and intensifies respiration [3]. Out of all the preparations researched, diquat shows the greatest effect on plant tissues; following the Reglone treatment, in case of higher rainfall, there are observed a repeated tissue moistening and a sudden fungal infection. Dimetiphine, however, is a typical growth regulator which acts slowly and does not destroy the plant tissue. Similarly etephon remains non-invasive – however there seems to be no literary coverage on the impact of those substances on the white lupin seed health status.

In the present research the treatments and preparations applied affected the seed health status of both cultivars considerably. Infected 'Bardo' (Fig. 1) and 'R-141' (Fig. 2) seeds obtained from control as well as from two-stage harvest plants were dominated by three fungal species: *Cladosporium herbarum*, *Colletotrichum gloeosporioides* and *Alternaria alternata*, while following the dimetiphine, etephon and diquat application, the number dropped to two: *C. herbarum* and *C. gloeosporioides*. Healthy seeds of both cultivars, irrespective of the treatment applied, were infected mostly by *C. herbarum* and of self-completing cultivar also with *B. Cinerea*. Dimetiphine, etephon and diquat decreased the *Fusarium* spp. occurrence considerably, both on healthy and infected seeds.

Fig. 1. Impact of selected agronomic practices on the total number of isolates on infected and healthy seeds of 'Bardo' white lupin harvested over 1996-1998

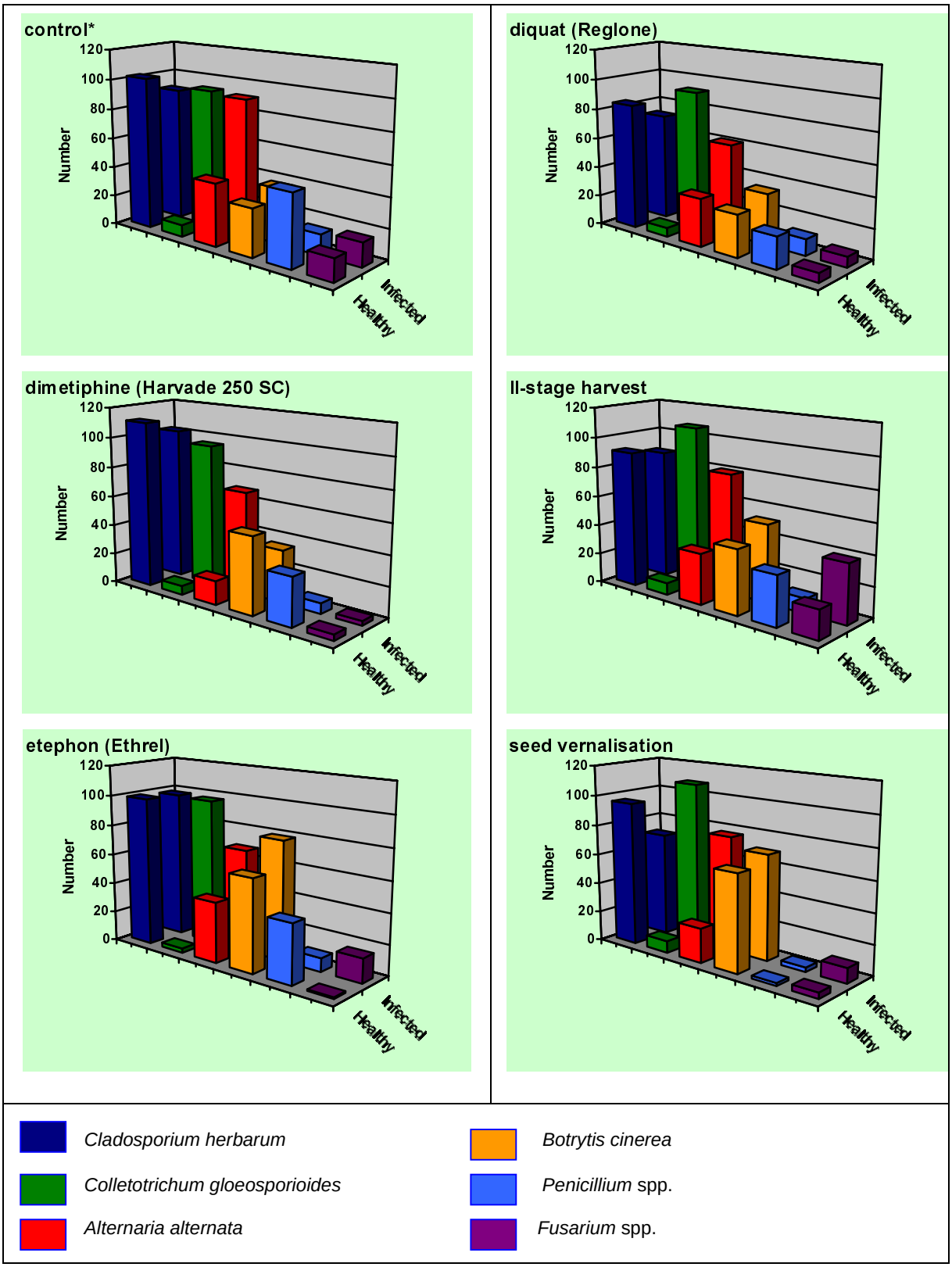


Cladosporium herbarum
Colletotrichum gloeosporioides
Alternaria alternata
Botrytis cinerea
Penicillium spp.
Fusarium spp.

*Total number of isolates: 18 species

PRINT

Fig. 2. Impact of selected agronomic practices on the total number of isolates on infected and healthy seeds of 'R 141' white lupin harvested over 1996-1998



*Total number of isolates: 18 species

CONCLUSIONS

1. White lupin seeds were identified to be infected with 18 fungal species; the 'R-141' self-completing cultivar seed yield contained significantly fewer seeds with fungal infection symptoms, as compared with 'Bardo' traditional cultivar.
2. Favourable-to-seed-health-status treatments included sowing material vernalisation and etephon for both cultivars and dimetiphine for 'R-141.
3. The two-stage harvest of both cultivars deteriorated the seed health status considerably; higher seed infection could have been due to a slow seed desiccation interchangeably with moistening when accompanied by high rainfall.
4. The fungal composition and number of isolates on both cultivar seeds were similar. The infected seeds of both cultivars were dominated by *Colletotrichum gloeosporioids, Alternaria alternata and Cladosporium herbarum,* while healthy seeds by *Cladosporium herbarum.*
5. Dimetiphine and diquat limited the number of dominant species to *C. herbarum* and *C. gloeosporioides,* while the two-stage harvest increased the number of dominant species by two, *Botrytis cinerea* and *A. alternata,* especially in 'R-141'.

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