

Impact of flower age and colour on infection of bean and alfalfa by *Sclerotinia sclerotiorum*

C.Y. Olivier, B.D. Gossen, and G. Séguin-Swartz

Abstract: *Sclerotinia sclerotiorum* is the causal agent of white mold of common bean (*Phaseolus vulgaris*) and blossom blight of alfalfa (*Medicago sativa*). No resistant cultivars of bean and alfalfa are currently available to growers in Canada. Studying physiological resistance is an important first step toward assessing and developing resistance in commercial cultivars. Flowers are generally the first tissue to be colonized by *S. sclerotiorum* in bean and alfalfa, and petal age has recently been shown to affect infection by *S. sclerotiorum* in canola (*Brassica napus*). Therefore, the development of the pathogen on detached flowers of bean and alfalfa was examined, focusing on the impact of flower colour and age on ascospore germination and mycelial growth. The colour and age of bean flowers did not affect ascospore germination or mycelial growth. Germtube growth was faster in extracts of 1-day-old petals than in extracts of 7-day-old petals for some cultivars. Petal extracts of bean had no inhibitory effect on ascospore germination and fungal growth, regardless of petal age and colour. Coloured flowers (purple or yellow) of alfalfa were colonized more slowly than white flowers. There was no difference in ascospore germination or mycelial growth in aqueous extracts from alfalfa petals of different colour, age or number. Petal age and colour did not have a substantial effect on the germination and development of *S. sclerotiorum* in alfalfa and bean.

Key words: white mold, *Phaseolus vulgaris*, bean, blossom blight, *Medicago sativa*, alfalfa, flower colour.

Résumé : *Sclerotinia sclerotiorum* est l'agent causal de la pourriture à sclérotés chez le haricot commun (*Phaseolus vulgaris*) et de la pourriture des corolles chez la luzerne (*Medicago sativa*). À l'heure actuelle, les producteurs canadiens ne disposent d'aucun cultivar résistant de haricot et de luzerne. L'étude de la résistance physiologique constitue une première étape importante dans l'évaluation et le développement de la résistance des cultivars commerciaux. Les fleurs du haricot et de la luzerne sont habituellement les premiers tissus à être colonisées par *S. sclerotiorum*, et l'on a récemment montré que l'âge des pétales a une incidence sur l'infection causée par *S. sclerotiorum* chez le canola (*Brassica napus*). Par conséquent, la croissance de l'agent pathogène sur des fleurs qui avaient été cueillies sur des plants de haricot et de luzerne a été examinée. L'examen a porté sur les effets de la couleur et de l'âge des fleurs sur la germination des ascospores et la croissance mycélienne. Pour certains cultivars, la croissance des tubes germinatifs a été plus rapide dans les extraits de pétales vieux d'une journée que dans ceux vieux de sept jours. Les extraits de pétales de haricots n'avaient aucun effet inhibiteur sur la germination des ascospores et la prolifération fongique, et ce, indépendamment de l'âge et de la couleur des pétales. Les fleurs violettes ou jaunes de la luzerne ont été colonisées plus lentement que les blanches. Il n'y a pas eu de différence en ce qui a trait à la germination des ascospores ou à la croissance mycélienne dans les extraits aqueux de pétales de luzerne, peu importe leur couleur, leur âge ou leur nombre. L'âge et la couleur des pétales n'ont pas vraiment influencé la germination et le développement de *S. sclerotiorum* chez la luzerne et le haricot.

Mots-clés : pourriture à sclérotés, *Phaseolus vulgaris*, haricot, pourriture des corolles, *Medicago sativa*, luzerne, couleur de la fleur.

Introduction

White mold, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is one of the most destructive diseases of bean (*Phaseolus vulgaris* L.) in temperate regions. White mold is

especially prevalent and destructive when bean crops are grown in relatively cool and moist environments (Steadman 1983; Huang et al. 1988) or under irrigation (Ferraz et al. 1999). Blossom blight, caused by *S. sclerotiorum* or *Botrytis cinerea* Pers.:Fr (Gossen et al. 1994; Holley et al. 1996),

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causes substantial losses in alfalfa (*Medicago sativa* L.) seed production across the Canadian prairies (Gossen et al. 1998). White mold and blossom blight are initiated by ascospores, which require an exogenous source of nutrients (usually senescent or injured tissue) prior to the infection of intact green tissues (Purdy 1958). On bean and alfalfa, the source of nutrients is generally senescent flowers (Steadman 1983; Huang et al. 2000), ascospore-contaminated leaves of bean in contact with moist soil, or bean leaves that have come in contact with sclerotia on the soil surface (Tu 1997). Mycelium spreads to the bean or alfalfa plant from colonized blossoms when humidity is high enough to provide the required duration of leaf wetness (Abawi and Grogan 1975; Lan and Gossen 1998).

White mold on bean cannot be managed effectively using a single approach. A combination of control measures are required to reduce crop losses, such as use of cultivars with physiological resistance (Steadman 1983) or disease avoidance (Schwartz et al. 1978; Saindon et al. 1995), cultural management that improves air-flow and minimizes humidity within the crop canopy, and foliar application of fungicides. Physiological resistance based on increased activity of plant defence-related enzymes (Miklas et al. 1993) or phytoalexin accumulation (Sutton and Deverall 1984) has been incorporated into elite breeding lines of snap bean (Dickson and Petzoldt 1994; Kmiecik and Nienhuis 1998).

Blossom blight in alfalfa is often managed with foliar fungicides during flowering (Gossen et al. 1999). Differences among alfalfa cultivars in disease reaction to *S. sclerotiorum* (Holley et al. 1996) and *B. cinerea* (Lan 1999; Lan and Gossen 1999) in controlled environments have been reported, but the mechanism underlying these differences has not been elucidated.

Flower characteristics may be associated with differences in disease reaction among bean cultivars. Flower colour in bean can vary from dark purple to white. Pigmentation is conditioned by multiple alleles at the *P* locus (Emerson 1909; Bassett 1992); colour intensity and distribution are dependent upon epistatic interactions of alleles at several other loci (Bassett 2003). Bean flowers contain anthocyanins (Nakayama 1964), which have been associated with disease resistance (Lee and Gould 2002), and the *P* locus is a candidate site for an enzyme involved in the flavonol and anthocyanin biosynthetic pathway (Bassett 2003). However, there was no clear relationship between flower colour in bean and resistance to *S. sclerotiorum* in previous trials (Hunter et al. 1981; Lyons et al. 1985). Flower age may also play a role. Ascospores of *S. sclerotiorum* readily infect senescent or dead blossoms of bean but do not infect freshly opened blossoms (Natti 1971).

Alfalfa cultivars are genetically heterogenous and exhibit a range of flower colours including dark purple, purple, yellow, and cream or white. Dark purple and purple flowers contain three anthocyanin pigments, whereas white flowers lack anthocyanins (Barnes 1966). The intensity of the purple colour is cumulative (i.e., dark purple flowers contain a higher concentration of the three anthocyanins than light purple flowers) (Barnes 1966). Yellow flowers contain primarily xanthophyll and carotene (Barnes 1966) and extremely winter-hardy lines (generally with a high proportion of *M. sativa* ssp. *falcata* in their background) have predomi-

nantly yellow flowers (Goplen and Gossen 1994). In 'Iroquois', a cultivar with purple- and white-flowered plants, incidence of infection by *S. sclerotiorum* was lower on the purple-flowered plants than on the white-flowered plants (Lan and Gossen 1998).

Detached leaf assays using mycelium of *S. sclerotiorum* grown on agar plugs have been used to assess the resistance of bean cultivars (Steadman et al. 1997; Kull et al. 2003). In the present study, inoculation of fresh detached leaves was performed using ascospores placed on petals, approximating the infection process under natural conditions. Because *S. sclerotiorum* generally colonizes senescing flowers first in bean and alfalfa, detached flower assays were conducted and the antifungal potential of aqueous extracts of fresh and senescent petals was examined. This work was conducted to evaluate the hypothesis that flower colour and age influence disease development of *S. sclerotiorum* in bean and alfalfa.

Materials and methods

Plant materials

Bean cultivars 'I92919', 'A55', 'G122', 'TR64.048', 'Benton' and 'Labrador' were selected for this study to provide a range of flower colour and disease reaction to white mold. Seed was provided by K. Kmiecik (Asgrow Vegetable Seeds, DeForest, Wis.). 'I92919' has white flowers and a strong physiological resistance to white mold (Rand et al. 1998; Kolkman and Kelly 2000). The pink-flowered cultivar 'G122' and a white-flowered line 'TR64.048' derived from 'G122' have been reported to have physiological resistance (Kmiecik and Nienhuis 1998; Kolkman and Kelly 1998). 'A55' is a susceptible short-vine cultivar with pink flowers (Park 1993; Steadman 1995). White-flowered 'Benton' and yellow-flowered 'Labrador' are susceptible to white mold (Miklas et al. 1999, 2006). Ten plants each of 'A55', 'G122', and 'Benton'; eight plants of 'Labrador'; and six plants each of 'TR64.048' and 'I92919' were grown in soil-less mix (peat moss, vermiculite, and sand, 2:2:1, v/v/v) plus slow-release fertilizer (Osmocote, Scotts-Sierra Horticultural Products Co., Marysville, Ohio) and maintained under a 16 h light : 8 h dark photoperiod and 22:16 °C day:night temperatures.

Alfalfa plants with uniform flower colour were selected for the study: two plants of 'Vernal' with dark purple flowers, two plants of 'Beaver' with purple flowers, two plants of 'Beaver' with white flowers, and two plants of 'AC Nordica' with yellow flowers. The cultivars are all moderately susceptible to blossom blight (Lan and Gossen 1998). Each plant was maintained in a 15 cm diameter pot filled with soilless mix under a 16 h light : 8 h dark photoperiod and 20:15 °C day:night temperatures.

Preparation of inoculum

Apothecia of *S. sclerotiorum* clone 321 (Kohli et al. 1995) were produced as described previously by Lefol (1998). To collect ascospores, a drop of sterile deionized water was placed on a sterile glass microscope slide that was quickly inverted over a puffing apothecium. Water drops containing ascospores were combined into sterile 1.5 mL microcentrifuge tubes and held for about 15 min at 4 °C. To prepare inoculum, ascospore concentration was es-

timated using a haemocytometer and adjusted to 5×10^4 ascospores/mL with sterile deionized water.

Infection assays on bean

The impact of flower colour and age on infection success was examined for each cultivar. To produce flowers and petals of a known age, all immature and opened flowers were removed from selected inflorescences, leaving only buds that would open within the same day. The inflorescences were bagged individually in glassine bags for 1 or 7 days to yield fresh (1-day-old) and senescent (7-day-old) flowers.

Detached-leaf assay

Ten leaves were collected from one plant per cultivar, mixed together, and randomly laid flat on moistened filter paper in plastic containers, 10 leaves per container. For each flower colour, a 1- or 7-day-old flower was placed on each of 10 leaves, and 9 flowers were each inoculated with a 10 μ L droplet of ascospore suspension (about 500 ascospores). A 10 μ L droplet of sterile deionized water was placed on the remaining flower. The containers were sealed with transparent plastic film to maintain high relative humidity (near 100%) and incubated at ambient temperature and daylength. Lesion size (largest length \times largest width) was estimated at 4, 5, and 6 dpi. The experiment was repeated three times.

Detached-petal assay

One- and 7-day-old flowers were prepared as described above. Ten flowers were collected per cultivar, flower colour, and age. The banner, wings, and keel (Simpson 2006) were dissected and placed individually on microscope slides. Each petal was inoculated with a 10 μ L droplet of ascospore suspension and placed in a humid chamber as described previously. Spore germination and germtube growth were stopped at 24, 48, and 72 h postinoculation (hpi) by immersion under a drop of lactophenol cotton blue. The samples were covered with a glass coverslip and examined using a microscope equipped with bright-field illumination. Germtube length was measured on a random sample of 10 germ tubes per petal. The number of appressoria per petal was estimated as 0 (no appressoria), <50 appressoria, 50–100 appressoria, and >100 appressoria. The experiment was repeated three times.

Infection assays on alfalfa

Inflorescences were trimmed and enclosed individually in glassine bags to provide fresh and senescent flowers, as described for bean.

Detached-leaf assay

Ten leaves were collected from one plant per cultivar, mixed together, and randomly laid flat on moistened filter paper in plastic containers, 10 leaves per container, as described previously. The banners (also known as the standards) of 1- and 7-day-old flowers were collected and placed on the detached leaves, one banner per leaf. Inoculation was as described for bean. Lesion size was measured at 4, 5, and 6 days postinoculation (dpi). The experiment was conducted three times.

Detached-inflorescence assay

Five 1- and 7-day-old inflorescences, each bearing three to eight flowers of uniform age, were collected from each donor plant. Inflorescences were placed in a humid chamber, and a 10 μ L droplet of ascospore suspension was placed on the uppermost flower of four of the five inflorescences. A 10 μ L droplet of sterile deionized water was used as the control (one inflorescence). The inoculated inflorescences were incubated under ambient conditions. The number of infected flowers per inflorescence was recorded at 6 dpi. The assay was conducted five times.

Detached-petal assay

The assay was conducted as previously described for bean.

Petal extracts

Bean

One- and 7-day-old flowers of each plant of the bean cultivars were obtained as described previously. Aqueous extracts were prepared by homogenizing 1, 2, 4, 8, or 12 petals in 0.5 mL sterile deionized water in an 2.5 mL Eppendorf microcentrifuge tube. Supernatants were filtered through 0.45 μ m nylon filters into Eppendorf tubes and placed on ice. For each sample, a water-repellent circular barrier (1 cm diameter) was drawn with a PAP Pen (Polysciences Inc., Warrington, Pa.) on a microscope slide and a 100 μ L aliquot of petal extract was mixed with a 10 μ L droplet of ascospore suspension inside the circle. Samples were incubated under ambient conditions in a humid chamber. Ascospore germination was arrested at 24 hpi by adding a drop of lactophenol cotton blue. Germtube length was measured on a random sample of 10 ascospores per sample, with five samples per plant of each cultivar and for each flower age. Ascospores were considered to have germinated if the germtube was at least as long as the spore (Kennedy et al. 1999). The experiment was conducted three times.

Alfalfa

Petal extracts for each flower colour (dark purple, purple, white, and yellow) and age (1- and 7-day-old petals) were produced as described above and ascospore germination in the extracts was examined as in bean. The experiment was conducted three times.

Data analysis

Analysis of variance was carried out for each assessment date in each study using the general linear models procedure in SAS (SAS Institute Inc. 2001). Homogeneity of variance was tested among the replications of each trial, and then the data were pooled for analysis. No white mold developed in the untreated controls in any trial, so the data for the controls were removed prior to analysis. In all experiments, means were compared using the least significant difference test at $P \leq 0.05$, except for the study on the number of appressoria on petals, where midpoints were used.

For alfalfa, there was no difference in the results among petal type (keel, wing, and banner) so the data were combined across petal type for presentation. Each of the three petal types was treated as a repetition of the trial.

Table 1. Percentages of leaves with lesions and mean lesion size in bean leaves inoculated with 1- and 7-day-old petals of different colour and age infested with ascospores of *Sclerotinia sclerotiorum* at 6 days postinoculation.

Cultivar	Flower colour	Leaves with lesions (%)		Leaf lesion size (cm ²)	
		1-day-old petals	7-day-old petals	1-day-old petals	7-day-old petals
'Benton'	White	75	63	22.5 b	28.0 c
'I92919'	White	80	100	3.6 a	4.6 a
'TR64.048'	White	75	75	24.2 b	18.1 b
'A55'	Pink	61	80	13.5 b	22.9 bc
'G122'	Pink	77	67	24.1 b	16.4 b
'Labrador'	Yellow	100	85	21.7 b	14.8 b
LSD _{0.05}		24.3	27.6	9.4	9.6

Note: Values in a column with the same letter are not significantly different ($P > 0.05$).

Table 2. Percentages of leaves with lesions and mean lesion size on detached leaves of alfalfa inoculated with 1- or 7-day-old petals of different colour and age infested with ascospores of *Sclerotinia sclerotiorum* clone 321 at 6 days postinoculation.

Cultivar	Flower colour	Leaves with lesions (%) ^a		Leaf lesion size (cm ²)	
		1-day-old petals	7-day-old petals	1-day-old petals	7-day-old petals
'Vernal'	Dark purple	37 a	47 a	0.60 b	1.35 ab
'Beaver'	Purple	50 b	67 b	0.61 b	0.44 a
'Beaver'	White	80 c	80 b	0.78 c	2.68 c
'AC Nordica'	Yellow	50 b	50 a	0.34 a	0.90 a
LSD _{0.05}		12.5	14.1	0.16	0.52

Note: Values in a column with the same letter are not significantly different ($P > 0.05$).

^aThirty leaves per flower colour and petal age.

Results

Assays on bean

Detached-leaf assay

At 4, 5, and 6 dpi, the number of detached leaves with lesions was similar among cultivars regardless of petal age (fresh or senescent) and colour. The same pattern was observed at all three dates, so only the results at 6 dpi are presented (Table 1). Significantly smaller lesions developed with flowers of 'I92919' as compared with flowers of the other cultivars when inoculated with 1- and 7-day-old petals (Table 1). For the other cultivars, lesions were smaller ($P \leq 0.05$) when flowers off of 'TR64.048', 'G122' and 'Labrador' were used compared with flowers of 'Benton', when 7-day-old petals were used (Table 1).

Detached-petal assay

All petals were infested at 24 hpi, regardless of cultivar, flower colour, or petal type (data not shown). At 72 hpi, all petals were covered with mycelium and appressoria numbers ranged from ~100 to 300 per petal with no significant differences among petal colours or age.

Assays on alfalfa

Detached-leaf assay

At 4, 5, and 6 dpi, there were fewer leaves with lesions when 'Vernal' (dark purple) flowers were used for inoculation as compared with the other flower types when the leaves were inoculated with 1-day-old petals. With 7-day-old petals, there were fewer leaves with lesions when 'Vernal' flowers and 'AC Nordica' (yellow) flowers were used for inoculation (data shown for 6 dpi; Table 2). At 6 dpi

Table 3. Percentages of infested flowers on detached 1- and 7-day-old inflorescences of alfalfa cultivars inoculated with ascospores of *Sclerotinia sclerotiorum* at 6 days postinoculation.

Cultivar	Flower colour	Infested flowers (%)	
		1-day-old inflorescences ^a	7-day-old inflorescences
'Vernal'	Dark purple	29 a	90
'Beaver'	Purple	29 a	75
'Beaver'	White	71 c	100
'AC Nordica'	Yellow	52 b	75
LSD _{0.05}		15.0	28.6

Note: Values in a column with the same letter are not significantly different ($P > 0.05$).

^aThirty inflorescences per flower colour and petal age.

only, leaves inoculated with 1- and 7-day-old white petals had developed larger lesions than leaves inoculated with dark purple, purple and yellow petals (Table 2).

Detached-inflorescence assay

Older inflorescences were more readily colonized than young inflorescences, and there were no differences among cultivars with older inflorescences (Table 3). For the young inflorescences, those with dark purple and purple flowers had the fewest infected flowers, followed by yellow inflorescences. White inflorescences were most readily colonized (Table 3).

Detached-petal assay

Petal type, whether a banner, wing or keel, had no effect on any response variable (data not shown). For 7-day-old

Table 4. Percentages of infested petals, mean germtube length, and number of appressoria on 1- and 7-day-old detached petals of alfalfa cultivars infested with *Sclerotinia sclerotiorum* at 24, 48, and 72 h postinoculation.

Cultivar	Flower colour	Infested petals (%) ^a			Germtube length (mm) ^b			No. of appressoria ^c		
		24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
1-day-old petals										
'Vernal'	Dark purple	43	52	48 a	0.79	1.10	>2.00	<50	<50	<50
'Beaver'	Purple	100	100	100 b	1.45	>2.00	>2.00	>100	50–100	50–100
'Beaver'	White	53	72	100 b	0.86	1.43	>2.00	0	50–100	>100
'AC Nordica'	Yellow	89	78	100 b	0.97	1.43	1.65	0	50–100	50–100
LSD _{0.05}		47	29.3	4.1	0.67	1.59	0.83			
7-day-old petals										
'Vernal'	Dark purple	89	89	100	1.24	>2.00	>2.00	>100	>100	>100
'Beaver'	Purple	81	82	100	1.38	>2.00	>2.00	<50	>100	>100
'Beaver'	White	59	100	97	1.44	>2.00	>2.00	>100	>100	>100
'AC Nordica'	Yellow	100	100	100	>2.00	>2.00	>2.00	>100	>100	>100
LSD _{0.05}		21.7	18.5	3.8	0.75					

Note: Values in a column with the same letter are not significantly different ($P > 0.05$).

^aNinety petals per flower colour and time of incubation.

^bMean germtube length per infested petal.

^cEstimated number of appressoria per infested petal.

Table 5. Mean length (mm) of germtubes on ascospores of *Sclerotinia sclerotiorum* incubated for 24 h in aqueous extracts of 1- and 7-day-old petals of bean cultivars.

Cultivar	Flower colour	No. of petals in extracts					LSD _{0.05}
		1	2	4	8	12	
1-day-old petals							
'Benton'	White	1.13 ^a	1.29	1.41	1.61	1.59	0.23
'I92919'	White	1.20	1.37	1.48	1.53	1.62	0.13
'TR64.048'	White	0.85	1.02	1.02	1.18	0.92	0.16
'A55'	Pink	1.36	1.63	1.71	1.60	1.97	0.12
'G122'	Pink	0.89	1.14	1.13	1.18	0.92	0.16
'Labrador'	Yellow	0.80	0.77	0.78	0.90	0.87	0.11
7-day-old petals							
'Benton'	White	0.46	0.59	0.87	1.15	1.24	0.10
'I92919'	White	0.30	0.35	0.42	0.61	0.90	0.10
'TR64.048'	White	0.78	0.91	1.03	1.38	1.35	0.06
'A55'	Pink	0.58	0.74	0.80	0.96	0.89	0.16
'G122'	Pink	0.98	1.03	1.29	1.43	1.32	0.11
'Labrador'	Yellow	0.72	0.86	1.09	1.32	1.44	0.09
LSD _{0.05}		0.09	0.11	0.11	0.14	0.11	

^aThirty samples of 10 ascospores per sample.

petals at 72 hpi, 97%–100% of the petals were infested regardless of cultivar or flower colour (Table 4). Petals were completely covered with mycelium and the number of appressoria ranged from 100 to ~300 (Table 4). For 1 day-old petals, there were significantly fewer infected dark purple petals than other petal colours at 72 hpi. Germtube length was similar at 72 hpi regardless of petal colour. Fewer appressoria were observed on 1 day-old petals as compared with 7 day-old petals at 24, 48, and 72 hpi. Petal color had no effect on number of appressoria.

Petal extracts

Germtube growth generally increased with increasing number of petals in the bean extracts, particularly with the 7-day-old petals (Table 5). Germtube growth was faster in

extracts of 1-day-old petals than in extracts of 7-day-old petals for 'Benton', 'I92919', and 'A55' (Table 5). The slowest germtube growth was observed in the extracts of one to eight 7-day-old petals of 'I92919' (Table 5).

In alfalfa, there were no differences in germtube length among cultivars, flower colour, or the age of the petals that were used to prepare the extracts. The only exception was extracts of eight and twelve 1- and 7-day-old petals of 'AC Nordica', where growth was higher than in the other treatments (Table 6).

Discussion

Sclerotinia sclerotiorum is pathogenic to more than 400 plant species (Boland and Hall 1994) and causes substantial

Table 6. Mean length (mm) of germ tubes on ascospores of *Sclerotinia sclerotiorum* incubated for 24 h in aqueous extracts of 1- and 7-day-old petals of alfalfa cultivars.

Cultivar	Flower colour	No. of petals in extracts					LSD _{0.05}
		1	2	4	8	12	
1-day-old petals							
'Vernal'	Dark purple	0.39 ^a	0.37	0.44	0.41	0.36	0.15
'Beaver'	Purple	0.37	0.41	0.44	0.51	0.39	0.10
'Beaver'	White	0.33	0.39	0.44	0.47	0.39	0.06
'AC Nordica'	Yellow	0.39	0.49	0.50	0.81	0.61	0.17
7-day-old petals							
'Vernal'	Dark purple	0.40	0.39	0.43	0.41	0.45	0.12
'Beaver'	Purple	0.35	0.35	0.42	0.47	0.42	0.09
'Beaver'	White	0.33	0.32	0.41	0.40	0.32	0.07
'AC Nordica'	Yellow	0.41	0.41	0.46	0.68	0.54	0.12
LSD _{0.05}		0.06	0.07	0.11	0.13	0.13	

^aThirty samples of 10 ascospores per sample.

yield losses to numerous crops, including bean and alfalfa. Fungicide application is the major method for management of the pathogen in bean and alfalfa crops. Bean cultivars that exhibit disease avoidance and (or) physiological resistance have been reported (Miklas et al. 2003), but commercial cultivars of beans with a high level of resistance are not available. Similarly, alfalfa cultivars are, at best, moderately susceptible (Lan and Gossen 1998). In both species, floral parts are important components in the infection process (Abawi and Grogan 1975; Huang et al. 2000).

The characteristic red, blue, and purple coloration seen in flowers is the result of anthocyanins in the alfalfa (Cooper and Elliott 1964) and of an association between anthocyanins and flavones in bean flowers (Robinson and Robinson 1931). An antifungal role for anthocyanins has been proposed in leaf tissue of four tropical species (Coley and Aide 1989) and *Brachystegia spiciformis* Benth. (Tuohy and Choinski 1990), but there is no information to our knowledge on the antifungal potential of petal anthocyanins. Flavones have been reported to occur as preformed antifungal compounds (see the review by Grayer and Harborne 1994) and as posinfection metabolites (Fawe et al. 1998).

Petal age also plays a role in susceptibility to infection in a number of plants. For example, in canola (*Brassica napus* L.), colonization of old petals occurs more quickly than on young petals (McLean 1958). Preliminary studies in canola and dog mustard (*Erucastrum gallicum* L.) showed that ascospore germination and germ tube growth were inhibited in aqueous extracts of 1-day-old petals and that the level of inhibition is positively correlated with the number of petals used to prepare the extracts (Lefol et al. 1997a, 1997b), suggesting that young petals contain antifungal substance(s).

There was no association between the colour of the bean flowers and ascospore germination or mycelial growth in our study, confirming previous reports (Hunter et al. 1981; Lyons et al. 1985). Fresh flowers were as easily colonized as senescent flowers. Petal extracts had no inhibitory effects on ascospore germination and fungal growth. The lesions that developed from inoculation with infested flowers of one cultivar ('I92919', white flowers) were smaller than from flowers of the other cultivars. This indicates that

flower colour per se in 'I92919' is not involved in physiological resistance to *S. sclerotiorum* (Rand et al. 1998; Kolkman and Kelly 2000). However, resistance in 'I92919' appears to be expressed in the flower tissue.

Young nonwhite alfalfa flowers were colonized more slowly than white flowers in contrast to the bean flowers. Lesions were smaller on leaves inoculated with nonwhite flowers, which may be the result of antifungal substances in these flowers. However, it is unlikely that these substances are anthocyanins or carotenoids, because ascospore germination and mycelium growth in the aqueous petal extracts were not associated with flower colour or the number of petals in the extracts.

Resistance to *S. sclerotiorum* in bean results from both physiological resistance and escape mechanisms (Boland and Hall 1987). Little is known about the biochemistry of the physiological resistance. In general, quantitative trait loci for physiological resistance in common bean are found in regions of the genome associated with general plant defense genes (Walter et al. 1990). Miklas et al. (1993) suggested that the activity of phenylalanine ammonia-lyase was involved in the partial physiological resistance of common bean. In our study, physiological resistance (as per published reports) was not associated with flower colour. More research is needed to determine physiological resistance in other plant parts.

Blossom blight of alfalfa was first identified in the western Canadian prairies in 1993 (Gossen et al. 1994). Development of epidemics is dependent on cool wet weather during flowering. In regions where blossom blight occurs frequently, reliable approaches for assessing the disease reaction of cultivars would be an asset. A previous study assessed the impact of flower orientation and colour in alfalfa, and found that upward-facing flowers had a lower incidence of blossom blight than downward-facing flowers. However, no differences were associated with flower colour (Lan and Gossen 1998). The difference between the studies may be the result of the use of different cultivars and inoculation protocols.

Studies on the nature of resistance to *S. sclerotiorum* are few in bean (Miklas et al. 1993; Sutton and Deverall 1984) and nonexistent in alfalfa. In both crop species, infection of

blossoms is a crucial step in disease development, so assessing resistance in blossoms is an important first step in the development of resistant cultivars. In alfalfa, mycelial spread was slightly slower in coloured flowers (purple or yellow) than white flowers; however, flower colour had no impact on infection of bean, and petal age had little or no impact on infection in either species. We conclude that petal colour and age do not have a substantial effect on the development of *S. sclerotiorum* in these crop species.

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