## Effect of Lygus spp and Botrytis spp on faba bean (Vicia faba L.) seed quality – are there insect-pathogen interactions?

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Effect of *Lygus* spp and *Botrytis* spp on faba bean (*Vicia faba* L.) seed quality – are there insect-pathogen interactions?

Surinder Kaur\(^1, 2\), Patty Reid\(^3\), K. Neil Harker\(^3\), Scott Meers\(^4\), James Thomas\(^1\), Syama Chatterton\(^2\), and Hector Cárcamo\(^2\)

\(^1\)Department of Biological Sciences, 4401 University Dr W, Lethbridge, Alberta T1K 6T5, Canada

\(^2\)Lethbridge Research Centre, Agriculture and Agri-Food Canada, 5403-1 Ave. South, P.O. Box 3000 Alberta, T1J 4P4, Canada

\(^3\)Lacombe Research and Development Centre, Agriculture and Agri-Food Canada, 6000 C and E Trail, Lacombe, Alberta T4L 1W1, Canada

\(^4\)Crop Diversification Centre S 301, Horticultural Station Road E, Alberta Agriculture and Forestry, Brooks, Alberta T1R 1E6, Canada

**Abstract:** *Lygus* bugs and *Botrytis* fungal pathogen, the causal agent of chocolate spot in faba bean, can cause necrotic spots on faba bean seeds thereby reducing market value. Mid-pod stage is the most susceptible stage for chocolate spot development and *Lygus* infestation in faba beans. Therefore, we hypothesised that concomitant presence of *Lygus* spp. and *Botrytis* spp. might increase seed necrosis. Hence, the study was conducted to determine: (i) the spatial and local distribution of chocolate spot and *Lygus* spp. in central and southern Alberta; and (ii) association of chocolate spot disease severity and *Lygus* abundance. Chocolate spot and *Lygus* were present in all the counties surveyed. Chocolate spot had a negative association with *Lygus* abundance, but only the latter was significantly associated with seed necrosis. *Botrytis* spp. were

\(^2\)To whom correspondence should be addressed (e-mail:Syama.chatterton@agr.gc.ca).
frequently isolated from the seeds despite lack of expression of chocolate spot on the foliage. No significant effect of Lygus abundance on Botrytis isolation from seeds was found. Therefore, seed quality losses can occur both due to the fungal pathogen and the insect which likely occupy different niches influenced by microclimate. Economic thresholds and management strategies will be required to keep insect populations and disease progression under check.

**Key words:** Botrytis spp., Chocolate spot, Faba bean, Insect-fungal interaction, Lygus spp., Seed damage, Survey

**Introduction**

Faba bean (*Vicia faba* L.) is a major temperate grain legume cultivated on about 2.1 Mha world-wide (FAO 2017). Faba bean can enhance the productivity and sustainability of agricultural systems by fixing up to 300 kg N/ha annually (Smil 1999) through a highly efficient symbiotic association with *Rhizobium* bacteria. Faba bean cultivation holds significant potential to Canadian agricultural production systems. Global consumption of faba bean is over 4 million tonnes per year and around 20% of this demand (750,000 tonnes) is imported. The main exporting countries are Australia, the United Kingdom, France and Canada (MacGill et al. 2017). Since 1960, faba bean production has declined by 56% (Agegnehu et al. 2006). Part of this decline is attributed to various biotic (e.g., fungal pathogens, arthropods, viruses and parasitic weeds) and abiotic stresses.

Chocolate spot is an important and endemic foliar disease in all faba bean growing regions (Stoddard et al. 2010). *Botrytis fabae* Sard. (teleomorph: *Botryotinia fabae* J.Y.)
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Lu et T.H. Wu) and Botrytis cinerea Pers.: Fr. (teleomorph: Botryotinia fuckeliana Whetzel) cause chocolate spot (Elad et al. 2007), but B. fabae is the major cause of epidemics. Chocolate spot has two phases: aggressive and non-aggressive. During the non-aggressive phase, small and discrete reddish-brown lesions develop on the leaves. Under continuous high relative humidity, the disease becomes aggressive; lesions start coalescing causing blackening and partial defoliation (Park and Lopetinsk 1999). Yield losses from chocolate spot are around 60-80% on susceptible cultivars and total crop failure may occur under severe epidemic conditions (Bouhassan et al. 2004). On the stem, chocolate spot lesions are usually superficial or may occur as streaks (Gourley and Delbridge 1973) while on pods and seeds, a brownish blemish may appear (Richardson 2008). The most susceptible stage is during mid-pod. Disease-free seed and a four-year crop rotation are recommended for chocolate spot management (Park and Lopetinsk 1999).

Lygus spp. (Heteroptera: Miridae) are a polyphagous pest complex. For example, the tarnished plant bug (Lygus lineolaris, Palisot de Beauvois) has more than 328 plant hosts throughout North America (Young et al. 1986). Lygus spp. attack several crops including canola (Butts and Lamb 1991), seed alfalfa (Tingey and Pillemer 1977), and buckwheat (Wise et al. 2005) on the Canadian Prairies. Four species of Lygus can infest faba bean, alfalfa and canola in western Canada depending on the region: Lygus lineolaris (Palisot de Beauvois), L. borealis (Kelton), L. elisus (Van Duzee) and L. keltoni (Schwartz) (Carcamo et al. 2002). Adults emerge from overwintering during early June and start mating and feeding on meristematic tissues such as apical buds and fruiting bodies (Tingey and Pillemer 1977; Handley and Pollards 1993). This type of feeding
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habit can result in shedding of buds, flowers, and pods, which may affect yield (Elmore 1955).

*Lygus* feeding can also result in discolouration of the seed coat, hull perforations or seed pitting (Hagel 1978) and localized tissue wilting and necrosis (Handley and Pollards 1993) leading to quality loss in various crops. Severe damage due to *Lygus* feeding on flowers, buds and seed pods was observed in Lesquerella (*Physaria fendleri*), a potential new oil-seed crop (Naranjo et al. 2011). Severe infestations can cause 5-20% seed damage in dry bean in Canada (Goodwin 2005). Since faba bean is a late maturing crop, it becomes a potential host for *Lygus* after alfalfa hay or canola are harvested (Jones 1999). Management of *Lygus* spp. in various crops can be achieved with the application of insecticide (Hollingsworth, et al. 1997). However, repeated application of an insecticide in faba bean plots in central Alberta, Canada, did not improve seed quality (Dosdall et al. 2003).

Faba bean high tannin seed type is used mainly for human consumption while low tannin seed types are used for livestock feed. Tannins are anti-nutritive compounds that affect palatability and digestion in monogastric animals (Olaboro et al. 1981). Both seed types are often downgraded due to the presence of necrotic spots and hull perforation on the seed coat. Losses in faba bean seed quality can be high depending on the seed grading parameters used by importing and exporting bodies. To meet the Canadian export standards for Grade No. 1, the upper limit is less than 4% of seeds damaged including damage due to seed perforation (1%) and zero tolerance for rotted or mouldy seeds (Canadian Grain Commission). The damage inflicted by *Lygus* on faba bean seeds could be confounded due to the concomitant presence of *Botrytis* spp. and *Lygus*
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spp. under field conditions. Knowledge of the cause of necrotic spots can help develop suitable management strategies to reduce the seed quality loss either by managing the disease or the insect. Therefore, the objectives of the present field study were: (i) to determine spatial and local distribution of chocolate spot and *Lygus* in faba beans in central and southern Alberta and; (ii) determine potential associations between chocolate spot and *Lygus*, and with necrotic seed damage.

**Materials and Methods**

**Field Survey/ Monitoring, Sampling Method and Weather Data Collection**

Faba bean fields were surveyed to monitor *Lygus* spp. abundance, chocolate spot disease severity index and seed damage in central and southern Alberta. In 2015, 40 commercial fields were surveyed for the disease and 43 for insect abundance while in 2016, 22 commercial fields were surveyed for both the organisms. To eliminate edge effect, samples for both pests were collected 40-50 m from the field edge at 10 points 50 m apart in each field following an inverted-U pattern. The cultivar and type (tannin or zero tannin) was also recorded for each field. Meteorological data for the 2015 and 2016 faba bean cropping season (May-Sept.) was collected from each region surveyed and averaged for maximum and minimum temperature (°C), precipitation (mm) and relative humidity (%). The information was downloaded from the closest Alberta weather station ([http://climate.weather.gc.ca](http://climate.weather.gc.ca)). Soil zone information was obtained from Alberta soil information viewer available at Alberta Agriculture and Forestry ([https://soil.agric.gov.ab.ca/agrasidviewer/](https://soil.agric.gov.ab.ca/agrasidviewer/)).
Disease Survey

The BBCH (Biologische Bundesanstalt, Bundessortenamt and CHemical industry) scale for faba bean growth stage was adapted from Knott (1990). The BBCH scale was developed for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species. Chocolate spot disease severity was rated at 10 plants/site mid-pod, BBCH 205(1) from the top, middle and bottom of the canopy on a scale of 0-4 where: 0: no symptoms; 1: small, discrete lesions (2-3 mm), covering 1-2% of leaf surface, no defoliation; 2: some coalesced lesions, covering 2-5% of leaf surface, 1-2% defoliation; 3: large coalesced sporulating lesions covering 5-10% of leaf surface, 50% defoliation and 4: extensive lesions on leaves, stems and pods covering >10% of leaf surface, severe defoliation, heavy sporulation and blackening (modified from Tivoli et al. 2006). Leaf samples (5-10) were collected randomly from diseased plants for pathogen isolation, identification and pathogenicity testing. Bagged leaf samples were kept on ice in Styrofoam coolers, and then stored at 4 °C until fungal isolations were made. A disease severity index (DSI) was calculated for each of the ten sampling points within each field: DSI = 100 ΣDisease severity class × no. of plants in class × 100/ total number of plants assessed × maximum disease scale (Wheeler 1969). To measure the disease progression in a season, DSI at late pod, BBCH 207(1) and pod senescence BBCH 209(1) was measured during early August and early September, respectively from various locations in Lacombe in 2015, only.

Insect Survey
Insect sampling was performed with a standard 38 cm diameter sweep net (BioQuip, U.S.A). At each of the 10 sites per field, 10 walking sweeps were made, each one at an approximately 180° arc, with maximum canopy penetration to sample as many adults and nymphs as possible. All 10 bags, collected per field, were labelled and maintained in a -20°C freezer until processed. Insect surveys were conducted twice per season: (i) mid-pod stage (mid-late July at the same time and sites as the CS disease survey) and (ii) maturity (late August to early September). *Lygus* counts per 10 sweeps per field were used as replicates. All *Lygus* adults were identified to species using the keys provided by Schwartz and Footit (1998). In 2015, *Lygus* abundance was monitored at the bud stage (early June), late flowering to early pod (early-late July) and pod senescence (late August- early September) stage at 21 selected fields in Lacombe.

**Seed Collection and Damage Assessment**

Faba bean pods were sampled at the pod senescence stage. One pod was collected from the top, middle and bottom from each of ten plants for a total of 30 pods per site with 10 sites per field during September. Pods from different plant height at each of the ten sites were kept in separate bags. The sampling pattern and location were the same as that used in the insect and disease surveys. After storing the pods in paper bags in the lab at room temperature for up to 1 month, seed damage was measured on a scale of 0-4 where 0: necrotic spots covering 0-5% of the seed coat; 1: 6-25%; 2: 26-50% 3: 51-75% and 4: 76-100%. This damage is calculated as seed necrosis index (SNI) as described previously for DSI. Samples from one site in central Alberta (Smokey River) were excluded from seed damage assessment ratings due to fusarium growth.
Seed Microflora Isolation, Identification and Pathogenicity Testing

To determine the pathogens associated with chocolate spot and various fungi colonizing faba bean seeds, fungal isolations were performed by culturing on media plates. Diseased leaf sections were surface sterilized in 1% sodium hypochlorite for 30s while the seeds were surface sterilized in 5% sodium hypochlorite for 60 s. The material was rinsed with autoclaved distilled water 4-5 times and blotted dry on sterilized filter paper. Potato dextrose agar (PDA) supplemented with 3.75% streptomycin (200 µl/500ml PDA) was used for plating, fungal isolation and purification. Five seeds with high per cent damage from the top, middle, and bottom plant canopy levels were plated per PDA plate. The hyphal tips from the margins of the resulting colonies were cut with a sterilized cork borer and transferred to a Petri dish containing fresh PDA. Fungal isolates were maintained in 25% glycerol stock at -20°C. Isolates were identified according to the colony characteristics and microscopic characters described by Barnett and Hunter (1987). Different isolates were grouped into operational taxonomy units (OTUs) for pathogenicity testing and DNA barcoding. Fungal DNA was extracted using DNeasy® Plant Mini Kit (Qiagen, Toronto ON), according to manufacturer’s instructions. The ribosomal DNA internal transcribed spacer (ITS) region was amplified using the primer pair ITS1 and ITS4 (White et al. 1990) and sequenced by Genome Quebec sequencing services. Sequences were compared to NCBI database using a BLASTn search for identification to genus. The pathogenicity of the isolates was tested on the low tannin faba bean cultivar, ‘CDC Snowdrop’. Faba bean seedlings were inoculated with the test fungi at five-leaf stage by spraying the inoculum on the leaves in replicates of three. Leaves (3-4) were punctured with an orienteering punch to facilitate infection.
The seedlings were covered immediately with a plastic bag to create a moist chamber for 72-96 h. The infection was quantified based on the disease rating scale described above and calculated as average disease rating.

**Statistical Analysis**

The DSI (average of 10 plant subsamples per site with 10 sites per field) for each site was square root transformed to normalize the variables. Analysis of variance (ANOVA) at $\alpha = 0.05$ was performed to determine significant difference between the survey years and among different canopy levels. Three fields were excluded from the analysis because of hail and other damage. Throughout the study, *Lygus* abundance refers to the number of *Lygus* per 10 sweeps (average of 10 sites in a field), and includes all adults and juveniles (1$^{\text{st}}$ instar to 5$^{\text{th}}$ instar) for mid- and mature pod stages. *Lygus* counts were log transformed to satisfy the assumptions of ANOVA. Effect of cropping year, crop stage and *Lygus* growth stages (nymphs and adults) on *Lygus* abundance were tested using generalized linear mixed model on log transformed *Lygus* counts at $\alpha = 0.05$. Seed necrotic index (SNI) at each site was square root transformed to normalize the variables. Standard Least Squares analysis within the Fit Model platform was used to determine significant difference between the survey years and among different canopy levels using restricted maximum likelihood (REML).

Correlations among different variables were estimated by REML method at a 95% confidence interval and coefficient of determination, $R$, was considered significant at $\alpha = 0.05$. We also performed a correlation analysis to determine if SNI was related to the isolation frequency of *Botrytis* spp. from the seeds. Necrotic spots on the seeds were
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categorised based on blemish type (black, rusty or clean), hull damage (perforated or non-perforated) and size of the necrotic spot (no spot, small or large). Pearson Chi Square Test of homogeneity was performed to determine if response to the necrotic spots differs when *Botrytis* spp. was isolated as compared to when no *Botrytis* spp. was isolated from the symptomatic seeds. Fisher’s exact test was performed at α = 0.05. Nominal logistic regression fit was performed to estimate the probability of necrosis type due to three dominating fungi isolated from the seeds, *Botrytis* spp., *Alternaria* spp. and *Fusarium* spp. All statistical analyses were performed using JMP® 12.1.0 (SAS Institute Inc., 2016).

**Results**

**Meteorological Data for Survey Years**

The growing season (May-Sept.) in 2015 was dry and hot compared to wet and humid in 2016. In 2015, maximum air temperature, average precipitation and humidity were 30.2°C, 48.9 mm and 65.2%, respectively. Maximum air temperature, average precipitation and humidity were 28.0°C, 66.7 mm and 68.9% in 2016 (Table S1).

**Disease Distribution**

In both survey years, chocolate spot occurred in all 56 fields. DSI (%) on plant foliage ranged from 0.1- 69.5% and 1.4 - 41.1% in 2015 and 2016, respectively (Table S2). However, disease pressure was significantly higher in 2016 than in 2015 (F= 5.9, d.f. =1, 57, P = 0.01) (Fig. 1). The DSI varied significantly among canopy levels both in 2015 (F= 117.7, d.f. = 2, 72, P < 0.0001) and 2016 (F= 174.6, d.f. = 2, 40, P < 0.0001).
It was highest at the bottom of the canopy and lowest at the top (Fig. 1). The DSI was significantly higher in seeds with high tannin content (mean = 18.54) as compared to seeds with low tannin content (mean = 11.44; F= 7.9, d.f. = 1, 54, P = 0.006). DSI for the 6 sites in the Lacombe area was significantly higher at pod senescence (mean DSI = 20.9) than at late pod (mean DSI = 8.6) (F= 29.2, d.f. = 1, 10, P = 0.001). Two hundred and one fungi were isolated from diseased faba bean leaves over the two years (Table 1) and grouped in five genera: *Alternaria* spp., *Fusarium* spp., *Stemphyllium* spp., *Botrytis* spp., and other saprophytes. ITS sequencing was not variable enough for species identification for most of these genera. *B. cinerea* and *B. fabae* can usually be distinguished by conidia size, but presumptive *B. fabae* cultures did not readily sporulate. Therefore, for the purpose of this study, both species are referred to as *Botrytis* spp. Multi-locus genotype sequencing of *Botrytis* isolates is ongoing but was beyond the scope of this project.

*Botrytis* spp. were moderate to highly pathogenic with higher isolation per cent in 2016 than in 2015 (Table 1). *Alternaria* spp. were a weak pathogens while *Fusarium* spp. and *Stemphyllium* spp. were moderately pathogenic.

**Lygus Abundance**

*Lygus* were significantly higher in 2015 (F= 16.95, d.f. = 1, 60, P < 0.0001) than in 2016 (Table 2). Overall, the assemblage in central Alberta in both years was mainly composed of *L. lineolaris*, *L. keltoni* and *L. borealis* (Table 2, Table S3). In general *L. keltoni* was dominant in the southern counties, whereas *L. lineolaris* dominated the assemblage further north. Significantly more adults than nymphs were captured in the
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population, (F= 26.7, d.f. =1, 45, P < 0.0001). *Lygus* nymphs were more abundant than the adults at the mid-pod while *Lygus* adults were higher than the nymphs at crop maturity. Nymphs and adults peaked at crop maturity. *Lygus* counts were higher in low tannin cultivars (mean = 16.51) compared to high tannin cultivars (mean = 15.8; F= 1.8, d.f. =1, 62, P = 0.18). A significant interaction (F= 89.4, d.f. = 2, 42, P < 0.0001) between *Lygus* stage × Crop stage (bud, pod and maturity) was observed from twenty-one fields in Lacombe in 2015.

**Seed Microflora and Seed Damage**

Seed damage due to presence of necrotic spots was quantified as the seed necrotic index (SNI). It was significantly higher in 2015 than in 2016 (F= 30.9, d.f. = 1, 63, P < 0.001) (Fig. 2). SNI also differed significantly between levels of canopy both in 2015 (F= 33.82, d.f. = 2, 36, P<0.001) and 2016 (F= 36.3, d.f. = 2, 42, P < 0.001). It was highest at the top and lowest at the bottom. SNI was higher in low tannin seeds (mean = 20.74) than in high tannin seeds (mean = 19.2), but this difference was not significant (F= 0.1, d.f. = 1, 63, P = 0.71). Overall, 1310 and 883 fungi were isolated from the faba bean seeds collected during 2015 and 2016 survey, respectively (Table 1). Five highly damaged seeds were plated from each of the three canopy levels. *Alternaria* spp., *Fusarium* spp. and *Stemphyllium* spp., were weak to moderately pathogenic on faba bean seedlings with an average rating of 3.3, 2.5 and 3.1, respectively. *Botrytis* spp. were moderate to highly pathogenic with an average rating of 3.5.
A significant difference with respect to hull perforation was observed depending on *Botrytis* spp. isolation from faba bean seeds ($\chi^2(1, N = 870) = 4.98, P = 0.03$). Based on the Fisher’s exact test, proportion of *Botrytis* spp. isolation was lower from hull perforated (29.05) (Fig. 3a) seeds than the non-perforated (70.95) seeds (Fig. 3b-c). A significant difference in the blemish type $\chi^2(2, N = 870) = 167.45, P < 0.0001$ and spot size $\chi^2(2, N = 870) = 38.18, P < 0.0001$ was observed when *Botrytis* spp. were isolated or not isolated from the seeds (Fig. 4). We tested the probability of hull perforation when the three different genera of seed fungi were isolated solely or in combination with other fungi. *Alternaria* spp. were isolated most frequently (0.45) from the hull perforated seeds (Fig. S1). However, only *Fusarium* was isolated more frequently from non-perforated than the perforated seeds (LR$\chi^2(1, N = 870) = 4.38, P = 0.036$). *Botrytis* spp. (LR$\chi^2(2, N = 870) = 42.68, P < 0.0001$) and *Fusarium* spp were more prevalent in isolates from the rusty seeds than from any other seed damage category. Probability of large necrotic spots were significantly higher when only *Botrytis* spp. (LR$\chi^2(2, N = 870) = 9.09, P = 0.01$) or *Alternaria* spp. (LR$\chi^2(2, N = 870) = 13.89, P < 0.001$) were isolated from the seeds than in cases where multiple fungi were isolated from a seed.

**Disease-Insect Associations and Effects on Seed Damage**

A negative correlation was observed between DSI in the plant canopy and average number of *Lygus* /10 sweeps. Overall, average number of *Lygus* /10 sweeps present at mid-pod stage and, average number of nymphs/10 sweeps captured in the entire season had no significant correlation with DSI. Average number of total *Lygus* ($R = -0.4, P = 0.01$) at crop maturity and *Lygus* adults ($R = -0.45, P = 0.002$) decreased
significantly with increase in the DSI. The DSI was high at the bottom and low at the top of the plant canopy while the reverse was observed for SNI. SNI was significantly correlated with the DSI, estimated from bottom of the plant canopy ($R = -0.54$, $P = 0.002$). SNI increased significantly with the *Lygus* abundance ($R = 0.38$, $P = 0.002$). *Lygus* nymphs significantly correlated to SNI ($R = 0.56$, $P < 0.0001$) while the adults were marginally significant ($R = 0.24$, $P < 0.0001$). Overall *Lygus* captured at mid-pod significantly increased SNI ($R = 0.46$, $P < 0.0001$). However, no association of total *Lygus* on the SNI at the crop maturity stage ($R = 0.2$, $P = 0.12$) was observed. Isolation percentage of *Botrytis* spp. was significantly correlated to average DSI ($R = 0.41$, $P = 0.006$). However, no significant effect was observed for overall *Lygus* ($R = -0.1$, $P = 0.4$), adults ($R = -0.05$, $P = 0.7$), nymphs ($R = -0.22$, $P = 0.1$) or SNI ($R = -0.001$, $P = 0.99$) on *Botrytis* isolation. *Botrytis* isolation frequency did not differed between different canopy levels ($LRX^2(2, N = 1140) = 3.6$, $P = 0.17$).

**Discussion**

Results from the present study showed three main outcomes: (i) a negative association between CSD and *Lygus* abundance; (ii) wide geographic distribution of CSD and *Lygus* abundance and (iii) characteristic seed quality loss due to *Botrytis* infection and *Lygus* feeding. Based on the correlation analysis a negative association was observed between CSD and *Lygus* abundance. Interactions between plant pathogens and insect herbivores can be direct or indirect, usually mediated through their shared plant host (Tack and Dicke 2013). The resulting associations can have positive, negative or null impact on each other or their host, depending on the nature of
the interaction. Plant pathogens and herbivores can alter plant morphology, canopy stand and biochemistry (Stout et al. 2006). These alterations influence induction of defense signalling pathways and plant volatiles, which subsequently affect the abundance, fitness and performance of the microbe or insect (Stout et al. 2006). Correlative field studies between abundance of plant pathogenic microbes and herbivores can unravel underlying association and potential impacts on their host.

Our results showed that sites with higher disease levels tended to have lower Lygus levels. Pathogens can influence the abundance of herbivores by exerting selective pressure on herbivore preference for healthy or infected plants (Tack and Dicke 2013, Friedli and Bacher 2001). For example, in a correlative field study on abundance of phytophagous insects on creeping thistle, *Cirsium arvense* (L.) Scop. (Asteraceae) that were infected or not infected by the thistle rust, *Puccinia punctiformis* (Str.) Röhl. (Uredinales), the overall attack rates by endophagous insects were higher in uninfected plants as compared to infected ones (Kluth et al. 2001). However, individual insect species differed in their response. Our study relied on correlative field data, but since a potential interaction was observed, a feeding preference study under controlled conditions should be performed to validate this observation.

In this study chocolate spot was significantly higher in the lower canopy compared to the upper canopy. Previous studies also report that leaf age is a key factor determining faba bean response to *B. fabae* (Bouhassan et al. 2004). In contrast, *Lygus* adults on cotton spent significantly more time at the top of the canopy than nymphs (Rosenheim et al. 2004). These two organisms likely have different niche preferences influenced by canopy microclimate. Abundant new growth tissues and reproductive structures
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dominate the upper canopy and provide feeding and oviposition sites for insects like *Lygus* that exploit the meristematic tissues (Jackson 2003). In contrast, microclimate within the crop canopy is the key factor that determines disease development by influencing primary and secondary inoculum production, conidial dispersion, infection and pathogenesis (Elad et al. 2007). Generally, slow drying of the aerial parts and presence of high humidity in the lower portion of the plant canopy offered favourable conditions for chocolate spot development. Hence, *Lygus* and chocolate spot may be separated within the plant canopy. However, a major limitation in this study was that vertical distribution of the *Lygus* throughout the plant canopy could not be determined by standard sweep net sampling. Laborious visual observations are needed to determine *Lygus* distribution within the canopy of faba beans to test the hypothesis that these two organisms occupy different niches. Besides microclimate, broad weather factors play an equally important role in determining *Lygus* populations (Varis 1995) and chocolate spot spread and epidemics (Jarvis 1980; Elad et al. 2007).

Similar to the annual difference in *Lygus* abundance, SNI was significantly higher in 2015 than in 2016, and SNI was significantly correlated to *Lygus* abundance. In our study, we observed a peak in *Lygus* nymphs and adults at mid-pod stage. Therefore, crop-flowering to the mid-pod stage is most vulnerable to *Lygus* attack and damage. The peak in *Lygus* abundance observed during faba bean crop maturity can likely be attributed to adults that dispersed from adjacent crops. Since faba bean is a late maturing crop, adults can move from their initial host that had matured or had been harvested, particularly canola and alfalfa. *Lygus* have mandibular stylets for piercing and sucking (Handley and Pollards 1993) and their feeding injury has caused necrotic
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spots on blackeye bean seeds (Woodrow and Stevenson 1952). The damage caused to the seeds through feeding is primarily the result of biochemical stimulus from the insect’s salivary polygalacturonase and mechanical injury from stylet probing (Strong 1970). It is not clear which stage can inflict maximum damage, but nymphs, especially 3rd-5th instar, are more active feeders than adults (Zink and Rosenheim 2005).

Snodgrass (1998) also found that *L. hesperus* nymphs prefer developing cotton squares, whereas adults prefer the vegetative structures. Further studies characterizing the feeding behaviour of nymphs and adults in faba beans are required to determine threshold population levels.

In contrast to *Lygus* and SNI, an inverse relationship between chocolate spot and SNI was observed. Chocolate spot was highest at the bottom of the canopy while SNI was lowest at the bottom. However, fungal isolation data showed that seed infection due to *Botrytis* spp. increased with the increase in chocolate spot. *Botrytis* spp. were isolated more frequently from non-perforated than hull-perforated seeds (an indication of *Lygus* feeding), thus providing evidence that seed damage due to *Lygus* did not increase seed infection by *Botrytis* spp.

We categorised faba bean seed damage into three categories: blemish type, hull perforation and spot type. *Lygus* can cause seed damage through typical hull perforation of the seed coat while *Botrytis* tends to cause a rusty type of blemish to the seeds. The phenols released from wounds caused by *Lygus* feeding are oxidized to quinones (toxic) and ultimately to non-toxic polymers producing characteristic brown discoloration of wounded tissue (Levin, 1976).
Seeds with hull perforation were less infected with *Botrytis* but fungi such as *Alternaria* and other saprophytes were frequently isolated. Wounds on the plant tissues can provide a window for pathogen infection and colonisation of the tissues. However, *Botrytis* sp. isolation from faba bean seeds with necrotic spots or hull perforations suspected to be caused by *Lygus* feeding was insignificant. However, temporal differences on arrival of the herbivore or the microbial pathogen can influence impact on the host.

From the current study, it is unclear what occurs first: *Botrytis* infects seeds first which deters feeding by *Lygus*, or *Lygus* feeding induces defense responses which exclude or suppress *Botrytis* colonization. Herbivores can avoid oviposition on leaves infected by a fungal pathogen as an adaptive strategy to aid the fitness of the offspring (Niinemets et al. 2013), and *Lygus* has shown a preference for healthy fruits in strawberries (Wibe et al. 2014). Another hypothesis that could explain our results is that necrotrophic pathogens and wounding (boring and chewing) arthropods induce plant defences via the JA signalling pathway (Thaler et al. 2012). On the other hand, biotrophic pathogens and sucking arthropods induce plant defences via the SA signalling pathway (Stout et al. 2006). Therefore, under such interactions one parasite can prime defence against another resulting in less severe or antagonistic impacts on plants (Hauser et al. 2013). Since, *Botrytis* is a necrotrophic pathogen and *Lygus* are sucking arthropods, we may expect severe (synergistic) impacts of both on the seed damage. However, all necrotrophic pathogens initially have a biotrophic phase in which they asymptptomatically colonize the host tissues (Spanu 2012), which may explain the less severe (antagonistic) effects observed in our study.
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The field study we presented here is correlative, based on field-scale survey data and seed damage assessment. Thus, studies under controlled conditions to confirm the observed association between chocolate spot and Lygus would provide further information regarding the nature of this interaction. Furthermore, interactions between pathogens and herbivores can be confounded by plant-mediated effects such as activation of defense responses or emission of plant volatiles. Fungal infections can change the volatile composition of host plants which can alter the attractiveness of the host plant to insects (Biere et al. 2002, Shapiro et al. 2012). Understanding the mechanisms of such associations will help to design and formulate more effective crop-protection strategies against herbivores and disease pests.

In conclusion, chocolate spot and Lygus are widely distributed throughout central and southern Alberta, and pose a threat to faba bean production. Lygus feeding is the main cause of necrotic spots on the seeds. However, Botrytis spp. can also downgrade the seed quality under optimum weather conditions. The contrasting effect of Lygus and chocolate spot on seed damage, suggests management practices for both may be required depending on field conditions. Forecasting chocolate spot and Lygus population levels, based on weather factors coupled with information regarding eco-regions, host range, host distribution, topography and developing economic thresholds will be required to establish robust and sustainable management practices.

Acknowledgements

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Abbreviations

Alberta Climate Information Service (ACIS); Disease severity index (DSI); Food and Agriculture Organisation (FAO); Inter transcribed spacers (ITS); Likelihood Ratio (LR); Potato dextrose agar (PDA); Restricted maximum likelihood (REML); Seed necrotic index (SNI)

References


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https://doi.org/10.1016/j.tplants.2012.02.010


https://mc.manuscriptcentral.com/cjps-pubs
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Fig. 1. Chocolate spot disease severity index (% untransformed) at various levels (top, middle and bottom) in the plant canopy estimated from faba bean growing areas in central and southern Alberta in 2015 and 2016

Fig. 2. Seed necrotic index (% untransformed) at three different levels in the plant canopy during 2015 and 2016 survey

Fig. 3. (a) Hull perforated faba bean seed; (b) Non-perforated and rusty faba bean seeds; (c) Non-perforated and rusty faba bean seeds showing *Botrytis* sp. growth on potato dextrose agar plate

Fig. 4. Frequency of occurrence of types of damage characterized from faba bean seeds as a function of *Botrytis* presence
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Table 1. Total number of fungal species isolated from symptomatic faba bean leaves and seeds, and average disease ratings of the isolates in 2015 and 2016

<table>
<thead>
<tr>
<th></th>
<th>Symptomatic leaves</th>
<th></th>
<th>Seeds</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2015</td>
<td>2016</td>
<td></td>
<td>2016</td>
</tr>
<tr>
<td></td>
<td>Average Disease Rating^a</td>
<td></td>
<td>Average Disease Rating</td>
<td></td>
</tr>
<tr>
<td>n (Isolation %)</td>
<td></td>
<td></td>
<td>n (Isolation %)</td>
<td></td>
</tr>
<tr>
<td>Botrytis spp.</td>
<td>7 (6.31) 4.04 ± 0.32</td>
<td>21 (23.33) 3.75 ± 0.17</td>
<td>228 (17.40) 3.1 ± 0.2</td>
<td>233 (26.39) 3.8 ± 0.2</td>
</tr>
<tr>
<td>Ascochyta spp.</td>
<td>0 0</td>
<td>2 (2.22) 2 ± 0.1</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Alternaria spp.</td>
<td>80 (72.07) 1.8 ± 0.05</td>
<td>37 (41.11) 2.26 ± 0.1</td>
<td>598 (45.65) 3.3 ± 0.2</td>
<td>183 (20.72) ^b</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>7 (6.31) 2.5 ± 0.2</td>
<td>13 (14.44) 3.13 ± 0.2</td>
<td>374 (28.55) 3.02 ± 0.4</td>
<td>454 (51.42) 2.05 ± 0.5</td>
</tr>
<tr>
<td>Stemphyllium spp.</td>
<td>8 (7.21) 2.4 ± 0.07</td>
<td>7 (7.78) 3.7 ± 0.5</td>
<td>58 (4.43) 3.15 ± 0.2</td>
<td>5 (0.57) 3.15 ± 0.9</td>
</tr>
<tr>
<td>Others</td>
<td>9 (8.11) 1.5 ± 0.1</td>
<td>10 (11.11) 1.85 ± 0.2</td>
<td>52 (3.97) 2.3 ± 0.2</td>
<td>8 (0.91) 1.7 ± 0.3</td>
</tr>
<tr>
<td>Total isolates</td>
<td>111</td>
<td>90</td>
<td>1310</td>
<td>883</td>
</tr>
</tbody>
</table>

Note: ^aAverage Disease Rating on a scale of 1-5
^bPathogenicity test was not conducted for Alternaria isolates
Table 2. Total number of Lygus species adults and nymphs, and *Lygus* species adults in Alberta, Canada during 2015 and 2016 survey

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of counties (number of fields)</th>
<th>Crop stage</th>
<th>Growth stage</th>
<th>Lygus species</th>
<th>Lygus lineolaris</th>
<th>Lygus borealis</th>
<th>Lygus keltoni</th>
<th>Lygus elisus</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>10(21)</td>
<td>Bud</td>
<td>Adults</td>
<td>844</td>
<td>603</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nymphs</td>
<td>256</td>
<td>56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>19(41)</td>
<td>Flowering</td>
<td>Adults</td>
<td>740</td>
<td>274</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nymphs</td>
<td>2372</td>
<td>297</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>19(41)</td>
<td>Maturity</td>
<td>Adults</td>
<td>5064</td>
<td>3526</td>
<td>628</td>
<td>1122</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nymphs</td>
<td>367</td>
<td>1122</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2016</td>
<td>13(21)</td>
<td>Flowering</td>
<td>Adults</td>
<td>197</td>
<td>79</td>
<td>54</td>
<td>58</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nymphs</td>
<td>293</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2016</td>
<td>1(21)</td>
<td>Maturity</td>
<td>Adults</td>
<td>1157</td>
<td>900</td>
<td>58</td>
<td>157</td>
<td>13</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Nymphs</td>
<td>77</td>
<td>157</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
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Fig. 1.

140x114mm (300 x 300 DPI)
Fig. 2.

Average Seed Necrotic Index (%) by Year and Canopy Level

Year / Canopy level

Bottom 2016  Middle 2016  Top
Bottom 2016  Middle 2016  Top

198x156mm (300 x 300 DPI)
Fig. 3.

67x50mm (300 x 300 DPI)
Fig. 4.

68x50mm (300 x 300 DPI)