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Field efficacy of *Bacillus thuringiensis galleriae* strain SDS-502 for the management of alfalfa weevil and its impact on *Bathyplectes* spp. parasitization rate

Govinda Shrestha[a], Gadi V.P. Reddy[a,*], Stefan T. Jaronski[b]

[a] Department of Research Centers, Western Triangle Agricultural Research Center, Montana State University, P.O. Box 656, 9546 Old Shelby Rd., Conrad, MT 59425, USA
[b] USDA-ARS-NPARL, 1500 N. Central Ave., Sidney, MT 59270, USA

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ABSTRACT

Alfalfa weevil, *Hypera postica* Gyllenhal, is an important pest in forage alfalfa worldwide, and especially so on the Northern Plains of North America. Neither the weevil-specific fungus, *Erynia phytonomi*, nor the weevil's parasitoids are able to routinely suppress outbreaks as they do in the eastern U.S. A new *Bacillus thuringiensis* var. *galleriae*, having a Cry8Da coleopteran-active toxin, has been recently commercialized. We examined the efficacy of this *B. thuringiensis* product against the *H. postica* in replicated field trials in north central Montana. Because it has been suggested that efficiency of the parasitoids, *Bathyplectes curculionis* and *Oomyzus incertus*, was inversely proportional to host numbers (i.e., parasitoid efficiency increased when host population is low), we also sought to determine if a partial reduction of larval *H. postica* populations with a *B. thuringiensis* would yield to greater parasitoid efficiency, manifested as higher percent parasitism among the surviving larvae. The *B. thuringiensis* gave 27–40% reduction in weevil numbers at the low label rate, 55–59% for the high label rate. Mean parasitism at the two research locations varied from 5–26% and 17–36% respectively, but application of the *B. thuringiensis* had no significant effect on parasitism levels, i.e. parasitism was not greater in treated than in carrier control plots.

1. Introduction

Alfalfa, *Medicago sativa* L. (Fabaceae), is a significant forage crop world-wide and in the U.S. yields 60 million metric tons of hay a year (White et al., 1995; USDA NASS, 2017). On the Northern Plains of the U.S. (Montana, North Dakota, South Dakota, Wyoming, and Nebraska), 2.18 million hectares were planted to alfalfa in 2016. It is very often grown as a perennial crop, being grown for 3–4 years. Alfalfa is the predominant forage of choice for dairy cows. It is also widely used to supplement range forage for beef cattle in the western U.S.

One of the more important pests of alfalfa is *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), the alfalfa weevil, also called the lucerne weevil. This curculionid beetle has a cosmopolitan distribution, but is considered an invasive pest in the U.S. While adults and larvae both feed on foliage, the larvae cause the majority of the damage, especially in the first cutting of the crop (Pellissier et al., 2017). Two annual cuttings are typical under irrigation in MT, with the first cutting having the greater economic value.

The main tool for minimizing damage from *H. postica* is early first cutting, although a number of chemical insecticides are registered for use if necessary. Insecticides are used on 25% of U.S. alfalfa acreage annually, with weevils being the main target (Gianessi, 2009). Insecticides targeting this insect in the western U.S. represented 33% of the total applied in the late 1990s (Radcliffe and Flanders, 1998). However, an increasingly important segment of alfalfa production is organic because cows producing organic milk must consume hay produced organically. National organic alfalfa production has grown from 45,800 ha in 2000 to 102,000 ha in 2011, the latest figures currently available (USDA ERS, 2011). In addition, many insecticides being used are extremely hazardous to pollinators, and probably have impacts on other beneficial insects in this system (Davis, 1970; Dumbre and Hower, 1977; Pitts-Singer and Barbour, 2017).

Hypera postica is considered a pest of foreign origin, first noticed in the western US in 1904 followed by eastern US in 1951, and across the nation by 1970s (Stoner, 1998). It has been the target of several classical biocontrol programs since the early 1900s. USDA introduced and emphasized biocontrol programs by using several parasitic hymenoptera in 1911 and in the mid-1950s, respectively. As a result, at least, ten parasitoid species have become established in the U.S. depending on the region: *Anaphes luna* (Girault) and *Patasson luna* (Girault)

* Corresponding author.
E-mail address: reddy@montana.edu (G.V.P. Reddy).

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(Hymenoptera: Mymaridae); *Dibrachoides druso* (Forester) and *Peridesmia discus* (Walker) (Hymenoptera: Pteromalidae); *Bathyplectes anurus* (Thomson), *B. curculionis* (Thomson) and *B. stenostigma* (Thomson) (Hymenoptera: Ichneumonidae); *Microctonus aethiopoides* (Loan) and *M. colesi* (Drea) (Hymenoptera: Braconidae); and *Oomyzus* (=*Tetrastichus*) *incertus* (Ratzeburg) (Hymenoptera: Eulophidae) (Bryan et al., 1993; Radcliffe and Flanders, 1998). Surveys done by the USDA-ARS in the past decade documented the establishment of two of the parasitoids in Montana and North Dakota, *B. curculionis* and *O. incertus* (Bryan et al., 1993). *Bathyplectes curculionis* has also been reported from Wyoming and Colorado (Pellissier et al., 2017). These parasitoids can often kill more than half the weevil larvae in a field. Thus, they can have an important role in helping keep numbers low in many years and locations. In contrast, a collaborative four-state survey across Montana, North Dakota, Wyoming and South Dakota revealed that the *M. aethiopoides* and *M. colesi* parasitoids attacking weevil adults are absent from the region (Rand et al., unpublished). One species in particular, *M. aethiopoides*, considered one of the most effective agents in other areas of the U.S., (Kingsley et al., 1993), is well established in Minnesota but is not present in the Northern Plains. The larval parasitoids, *B. curculionis* and *O. incertus*, however, parasitized 4 − 85% and 0–16% weevils, respectively in a 2010–11 survey of Montana fields, while *B. anurus* and *B. stenostigma* were absent (Rand, 2013).

The fungus *Erynia (=Zoophthora) phytonomi* (Arthur) (Zygomycetes: Entomophthoraceae), specific to *H. postica*, can be a major mortality factor of larval alfalfa weevil (Hostetter et al., 1983). This fungus first appeared in Ontario, Canada in 1973, but has spread extensively and now commonly attacks weevil larvae throughout the eastern and Midwestern U.S.; it is not known to occur outside North America. In a study of natural enemies of *H. postica* in Virginia, the fungus was present in 82% of sampled fields with an in-field prevalence of 11–50% (Kuhar et al., 1999). However, this fungus seems to be rare in northeastern Montana and northwestern North Dakota, based on surveys conducted 2010–16 in alfalfa throughout the region (Jaronski and Rand, unpublished). The reasons for this disparity are unclear. The entomopathogenic fungus, *Beauveria bassiana* sensu lato (Balsamo) Vuilemin (Hypocreales: Clavicipitaceae), has been described from *H. postica* (Hedlund and Pass, 1968). In surveys during 2010–16 through northeastern Montana, this fungus was generally uncommon (Jaronski unpublished data). Reddy et al. (2016), in evaluating commercial biopesticides based on *B. bassiana* and *Metarhizium brunneum* (*anisopliae*) (Metsch.) (Hypocreales: Clavicipitaceae), as well as several biorational materials, observed only a commercial spinosad *Saccharopolyspora spinosa* (Mertz & Yao) (Actinomycetales: Pseudonocardiaceae) had satisfactory efficacy in laboratory bioassays; the fungi had little to no efficacy. No field trials were reported.

Bacillus thuringiensis Berliner or Bt (Bacillales: Bacillaceae) has been developed for a wide range of targets—Lepidoptera, Coleoptera and Diptera (Bravo et al., 2011). This Gram positive bacterium, produces insect-toxic protein inclusions during sporulation. These Insecticidal Crystal Proteins (ICPs), also known as Cry proteins or delta-endotoxins, are highly toxic to a wide variety of important agricultural and health related insect pests as well as other invertebrates but show great specificity based on variations in their amino acid sequence. Among the delta endotoxins, Cry3a was determined to be toxic for *H. postica* and several U.S. patents were issued regarding its use (Herrnstadt and Soares, 1989). Its commercial development never materialized. Abbott Laboratories explored the use of beta exotoxin of *B. thuringiensis* (Wilson et al., 1984; Hornby and Gardner, 1987) but never registered the compound, which is carcinogenic and teratogenic. A Cry6B protein was demonstrated in Iran to have high activity against *H. postica* (Sharma, 2011) but does not seem to have been developed further. In the early 2000s, a *B. thuringiensis* var. *galleriae* strain, SDS-502, producing a Cry8Da toxin active against several larval and adult Scarabeidae, was identified by Asano et al. (2003). This strain was subsequently shown to have activity also against adult emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) (Bauer and Londono, 2010). SDS-502 has been commercialized in the U.S. and Canada by Phyllom Bios (Oakland CA, USA) as Beetle-Gone®, targeting a range of larval and adult Scarabeidae (Phyllom BioProducts, 2017). Activity against larval *H. postica* was first documented by the company in sponsored field trials in California (Godfrey et al., 2014). This new material presents a potentially useful tool to manage *H. postica*. It is also certified as organic in the U.S.

One of our goals was to determine the efficacy of a commercial formulation of SDS-502 against the *H. postica* under Montana conditions. Rand (2013) suggested that efficiency of *B. curculionis and O. incertus*, the predominant parasitoids in Montana alfalfa, was inversely proportional to host numbers, parasitism being density independent. Therefore, we wanted to determine if a partial reduction of larval *H. postica* populations with *B. thuringiensis* would yield to greater parasitoid efficiency, manifested as a greater percent parasitism among the surviving larvae. If there would be synergistic or additive relationship between the microbial and the parasitoids, overall *H. postica* population suppression would be greater.

2. Materials and methods

2.1. Locations of alfalfa field trials

The research reported here was conducted in 2016. The experiments were conducted at two locations: Valier (N 48° 35.192 W112° 21.169) and Conrad (N 48° 30.206 W112° 14.350), Pondera County, Montana, USA. Both alfalfa fields had reached economic threshold level (1 larva/stem), as determined by larval numbers prior to field selections. Ages of the crop ranged from 3 to 5 years and the area of the Valier and Conrad fields were 68 and 16 ha, respectively. Alfalfa was grown according to recommended industry standards and both were irrigated fields.

A randomized complete block design (RCBD), with four replicates per treatment, was used. Each treatment plot was 6 × 6 m, and separated from each other by 3 m buffer zones to avoid any overlap of treatment effects. Plots were situated at least 6 m inside field edges.

2.2. Bacillus thuringiensis galleriae SDS-502 application

A commercial formulation of *B. thuringiensis galleriae STS-502* (BeetleGone®) (76.5% a.i., > 0.85×10^{10} CFU/g) was provided by Phyllom BioProducts Corporation, Oakland, California, USA. The low and high recommended application rates of BeetleGone corresponding to 2.2 and 4.4 kg, respectively, per hectare in 234 L ha^{-1} were used for experiments. NuFilm® 17 (Miller Chemical and Fertilizer, LLC, Hanover, Pennsylvania, USA) was added to each BeetleGone treatment (583 ml ha^{-1}) as a sticker. The spray suspension was prepared by mixing the product materials with water, followed by addition of the NuFilm17, and agitated well before spray application. The diluted NuFilm 17 served as a carrier control treatment.

Treatments were applied using a CO_2-pressurized backpack sprayer (pressure of 275 kPa) calibrated to deliver 252 L ha^{-1} through a two-person, 3.66 m, boom with TeeJet® TP8002VK nozzles (Spraying Systems Inc., Wheaton IL, USA) spaced 0.46 m apart. Each plot was sprayed in two swaths. The plots were sprayed on June 14, 2016, and alfalfa plants were about 70–80 cm at the time of spraying. The spraying activity was performed between 6 and 8 am local time.

2.3. Sampling

2.3.1. Alfalfa weevil larvae population

Hypera postica larvae were sampled in all plots to determine the treatment effects. Sampling was conducted 2 days before treatment application, and 3 and 7 days after applications. Ten samples were taken from each treatment plot, with 3 alfalfa stems/sample, and the sampling was performed along an N-shaped transect beginning 1 m into

the plot. Alfalfa stems were cut from the base of the plant with scissors, placed into one zipper-lock bag, and kept in a picnic cooler. The samples were returned immediately to the lab and larvae dislodged from foliage by vigorous shaking in a plastic bucket. The larvae were categorized into two age classes-'young' (L1-L2) and 'old' (L3-L4).

2.3.2. Parasitization rate of Bathyplectes spp.

Parasitism by *Bathyplectes* spp. in *H. postica* larvae was assessed in treatment plots with larvae collected at 7 days post application. Both stem-cut and sweep net sampling were used. Sweeping was conducted with a standard sweep net (180° arc) with 20 sweeps in each treatment plot.

The larvae collected from each treatment plot were kept in plastic zipper-lock bags with some alfalfa foliage and transported immediately to laboratory. In the lab, *H. postica* larvae from each treatment plot were transferred into a large paper bag with a paper towel in the bottom. Fresh alfalfa foliage (1–2 healthy stems) was placed in each bag, and the top of the bag was folded multiple times and secured with a large binder clip. Fresh foliage was added every other day as needed and dried out foliage was left in a bag in order to avoid risk of losing insects. All bags were kept at room/lab temperature for 14 days at which time most insects had pupated or eclosed into adults. *Hypera postica* larvae parasitized by *Bathyplectes* spp. were determined by the presence of dark brown, football-shaped cocoons with white equatorial bands even with the surface of the cocoons (Tharp, 2015).

2.4. Data analysis

The data were analyzed with R 2.15.1 (R Development Core Team, 2011). For all data, a test with a normal quantile-quantile plot was performed to confirm normality of the data and equality of variance. Where appropriate, Tukey's contrast pairwise multiple comparisons were used to test for significant differences in means (Hothorn et al., 2008). Furthermore, the data were subjected to angular transformation prior to statistical analysis.

2.4.1. Alfalfa weevil population

The percentage reduction of alfalfa weevil population was calculated relative to the initial larval population (assessed 2 days before spraying) as follows:Alfalfa weevil density reduction (AWDR) (%)

$$= \frac{AWDRst0 - AWDRst1}{AWDRst0} \times 100;$$

where $AWDR_st_0$ represents the number of *H. postica* larvae recorded at each treatment plot before the BeetleGone application and $AWDR_st_1$ is the number of *H. postica* larvae recorded at each treatment plot in each sampling time (3 days or 7 days after BeetleGone or carrier control treatments) (Shrestha et al., 2015).

The overall data were fitted to a linear mixed model with sampling time interval, SDS-502 rate and *H. postica* populations per replicate as fixed effects (categorical variables converted to factors), the variation in *H. postica* populations (1|Unit) as random effect and the mean *H. postica* populations per treatment as response variable using the function "lmer". The mean alfalfa weevil population per treatment was calculated using the "Summaryby" work package (doBy). The model was then simplified with stepwise removal of factors having no effect. The Kenward-Roger test was run using the function "KRmodcomp" to compare the models (Halekoh and Højsgaard, 2012). One-Way Analysis of Variance (ANOVA) was carried out to determine the effect on *H. postica* population across treatment levels at each sampling time including pre-treatment data.*H. postica* percent control level due to SDS-502 rates was further calculated by using the formula given by Henderson and Tilton (1955):

$$= 100\left[1 - \frac{Ta \times Cb}{Tb \times Ca}\right]$$

Here T*b* is the number of *H. postica* larvae collected per sampling unit before treatment, T*a* the number collected after treatment, C*b* the number collected from the carrier control plot before treatment, and C*a* the number collected from the carrier control plot after treatment of test plots. *H. postica* percent control level data were analyzed with a similar method as described for AWDR. The further consideration of Henderson calculation was mainly because it provides conservative estimates of control efficiency, especially in the alfalfa fields when the numbers of larvae decrease naturally over time in both treated and untreated plot.

2.4.2. Parasitism

One Way-ANOVA was performed to evaluate whether the spray of SDS-502 had an effect on parasitism levels by *Bathyplectes* spp. The parasitism percentage was calculated as numbers of parasitoid pupae formed/total number of *H. postica* larvae reared from each plot × 100. Linear regression was further used to analyze the correlation between mean parasitism percentage and mean 7 days weevil larvae reduction percentage after the treatment application through stem-cut method. Data from Valier and Conrad locations were pooled for analysis.

3. Results

3.1. Effects on alfalfa weevil populations

The overall mean number (± SE) of *H. postica* larvae per 30 stems 2 days before the treatment applications at the Valier and Conrad locations ranged from 11.25 to 12.75 and 10.75 to 13.00, respectively, across all plots (Fig. 1). No significant differences were found between pre-treatment data at both locations (Valier: $F = 0.45$; $df = 2, 9$; $P = 0.65$; Conrad: $F = 0.84$; $df = 2, 9$; $P = 0.46$). Mean number of post-treatment *H. postica* larvae per 30 stems (± SE) for Valier location varied from 8.00 ± 0.40 to 13.00 ± 1.58 and 5.25 ± 0.85 to 11.75 ± 0.85 respectively, across all treatments at the 3 days and 7 days post-applications (Fig. 1). The corresponding values for Conrad location were 7.75 ± 0.25 to 11.75 ± 0.85 and 4.75 ± 0.95 to 9.50 ± 0.50 respectively. There were significant treatment effects for both SDS-502 rates (Valier: $F = 13.19$; $df = 2, 18$; $P < 0.0001$; Conrad: $F = 15.20$; $df = 2, 18$; $P < 0.0001$) and at both sampling times (Valier: $F = 6.09$; $df = 1, 18$; $P < 0.05$; Conrad: $F = 5.66$; $df = 1, 18$; $P < 0.05$). There were no interaction effects between treatments and sampling times (Valier: $F = 0.10$; $df = 2, 18$; $P > 0.05$; Conrad: $F = 0.06$; $df = 2, 18$; $P > 0.05$).

Across the treatment levels, significant differences in *H. postica* reduction percentage occurred at both 3 days post treatment (Valier: $F = 5.32$; $df = 2, 9$; $P < 0.05$; Conrad: $F = 6.78$; $df = 2, 9$; $P < 0.05$) and 7 days (Valier: $F = 8.93$; $df = 2, 9$; $P < 0.01$; Conrad: $F = 9.98$; $df = 2, 9$; $P < 0.01$). The percentage reduction of *H. postica* larval populations with the two SDS-502 treatments was rate-dependent. (Table 1). SDS-502 provided 27% and 40% reduction in weevil numbers at the low label rate at Valier and Conrad, respectively, and 55% and 59% reduction at the high label rate at the 7 days post-treatment (Table 1).

Based on the Henderson and Tilton (1955) correction, over both dates combined there was significant difference in *H. postica* control levels between two rates of SDS-502 at both Valier ($F = 9.46$; $df = 1, 12$; $P = 0.009$) and Conrad ($F = 10.71$; $df = 1, 12$; $P = 0.006$) locations. Sampling date was also a factor that significantly affected the *H. postica* control level at Valier location ($F = 6.43$; $df = 1, 12$; $P = 0.02$) but without effect at Conrad location ($F = 0.43$; $df = 1, 12$; $P = 0.52$). When control levels were compared at each sampling date, significantly higher weevil mortalities were recorded for the high rate of SDS-502 at 3 days post-application at both locations, while there was no difference at 7 days post-application (Table 1).

Mean *H. postica* control level ranged 12–32% for the low rate and 36–51% for the high rate of SDS-502 at 3 days and 7 days post-

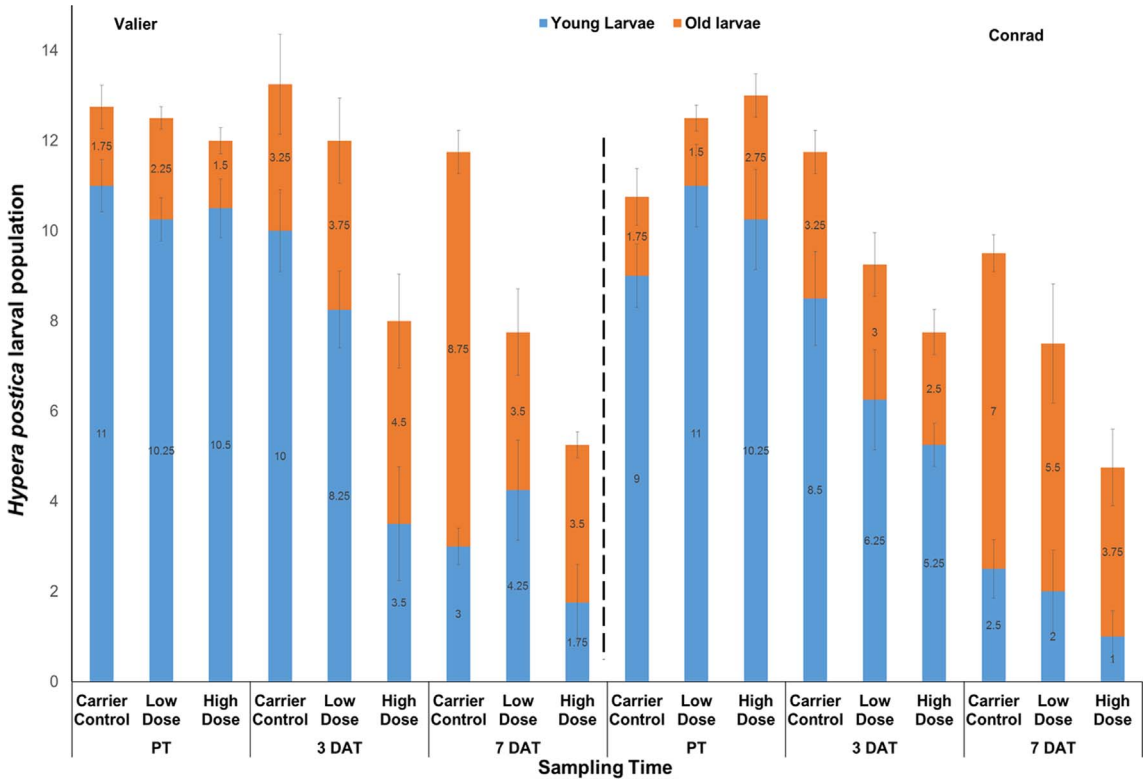

Fig. 1. Stacked bar chart of mean values (± SE) for *Hypera postica* larvae/30 stems in treatment category in sampling times at Valier and Conrad, Montana. The specific larval count are further presented inside bar. PT; Pre-treatment; and DAT; Days after treatment application.

applications, respectively, at the Valier location. At the Conrad location, average *H. postica* control level for the low rate of SDS-502 did not vary at 3 and 7 days post-treatment (31 and 32%) but did vary from 43 to 54% at the high rate (Table 1).

3.2. Parasitism level

Bathyplectes spp. cocoons were found after rearing of *H. postica* larvae collected from both experimental locations. The mean parasitism levels varied from 5–26% and 17–36% respectively at Valier and Conrad research sites (Table 2). There were no significant differences in rates of parasitism among treatments at both Conrad (stem cut: $F = 3.02$; $df = 2, 9$; $P = 0.09$ and sweep netting: $F = 0.87$; $df = 2, 9$; $P = 0.45$) and Valier (sweep net: $F = 2.20$; $df = 2, 9$; $P = 0.17$) with one exception. At Valier, in the stem-cut samples from high rate SDS-502 plots only, there was a significantly lower mean parasitism rate (5.0% ± 5.00) compared to all other treatments (Table 2). No significant relationship between parasitism levels and *H. postica* reduction percentage at the 7 days post-application was determined, based on R^2 value (0.336) as predicted by a linear regression equation (Fig. 2).

4. Discussion

Our results indicated that SDS-502 can be used as an alternative to synthetic pesticides for *H. postica* control, verifying the data from an earlier field study in California (Godfrey et al., 2014). The greatest mean *H. postica* control level recorded in both study locations (Valier and Conrad) compared closely with the 69% control in the California study. However, our treatment dose effects and time effects on *H. postica* levels contrasted to results recorded by the Godfrey et al. (2014), in that the *H. postica* control levels 3 days after treatment in our study were considerably less than half of the California levels, indicating possibly slower onset of toxicity. Lower environmental temperatures (14–16 °C) during the Montana test, compared to those in California (> 20 °C), may have reduced alfalfa weevil larval feeding activity (Mostafa et al., 2005), thus intake of the Bt (Katbeh-Bader et al., 1999).

The most successful parasitism (cocoon formation) usually occurs in intermediate stages of larvae since parasitoid eggs oviposited in matured larvae may not have enough time to hatch and mature before the larvae pupate (Duodu and Davis, 1974). Rand (2013) suggests that parasitoid *Bathyplectes* spp., particularly *B. curculionis* is relatively ineffective in controlling *H. postica* at high population level. Therefore,

Table 1
Cumulative percentage reduction (mean ± SE) of *Hypera postica* larval population on alfalfa plants after *Bacillus thuringiensis* SDS-502 or carrier control application.

Location	Sampling time	Treatment		
		Carrier control	Low dose	High dose
Valier	3 DAT	1.9 ± 1.92b	11.0 ± 6.88b (12.5 ± 7.65B)	33.2 ± 2.07a (35.7 ± 1.99A)
	7 DAT	9.2 ± 3.68b	26.8 ± 10.90b (31.9 ± 6.96A)	55.1 ± 8.29a (51.0 ± 8.99A)
Conrad	3 DAT	5.8 ± 5.77b	24.9 ± 5.07b (31.3 ± 4.64B)	38.3 ± 5.96a (43.5 ± 5.44A)
	7 DAT	14.2 ± 8.37b	39.6 ± 5.17a (31.6 ± 5.88A)	59.5 ± 10.90a (54.2 ± 12.34A)

Different letters within a row bearing same upper case or lower case letters indicate significant differences between treatments (Tukey test, $p < 0.05$). The values in parentheses denote the mean *Hypera postica* control levels calculated based on Henderson and Tilton correction (Henderson and Tilton, 1955).

Table 2
Mean percent parasitism (± SEM) by *Bathyplectes* spp. in *Hypera postica* larvae 7 days after *Bacillus thuringiensis* SDS-502 or carrier control application.

Location	Sampling method	Treatments		
		Carrier control	Low dose	High dose
Valier	Stem cut	19.3 ± 1.68a (8/44)	15.8 ± 2.17a (4/26)	5.0 ± 5.00b (1/21)
	Sweep net	25.8 ± 3.05a (23/87)	18. 2 ± 2.41a (15/79)	24.1 ± 2.84a (18/72)
Conrad	Stem cut	36.3 ± 5.54a (14/38)	22.4 ± 4.03a (7/30)	17.5 ± 6.85a (4/19)
	Sweep net	26.8 ± 5.28a (26/102)	20.2 ± 2.53a (20/98)	21.3 ± 3.62a (18/87)

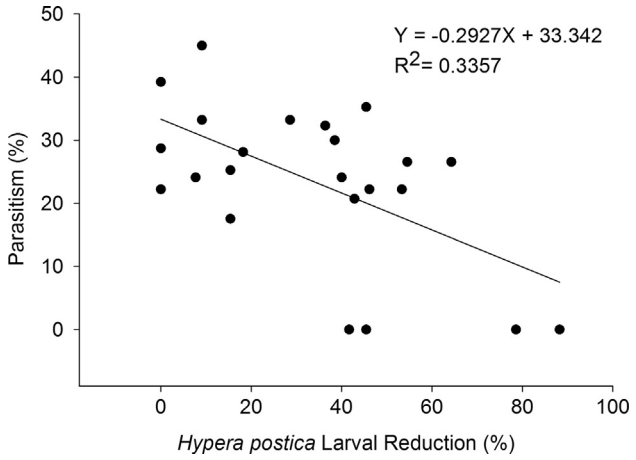

Fig. 2. Relationship between *Bathyplectes* spp. parasitism and *Hypera postica* larval reduction levels, 7 days post-application. Valier and Conrad locations data were pooled for analysis.

we hypothesized that a reduction in young aged larvae numbers would increase the observed percent parasitization by *Bathyplectes* spp. This situation did not occur. Rather, there was no significant correlation between reduction in larval population and percent parasitism (slope = −0.29). Larval population reductions as great as 55–59% did not yield a significant increase in the rate of parasitism in comparison to the parasitism prevalence in the much smaller reductions in larval populations in the carrier controls. At the same time, there was no reduction in parasitism by *Bathyplectes*, indicating that concomitant use of the two is compatible. A later application of Bt, targeting second-third instars that are the preferred hosts of the parasitoid, might interfere with successful *Bathyplectes* reproduction (Vinson and Iwantsch, 1980; Goh et al., 1989).

No previous reports are available on the effect of SDS-502 spray on parasitization rate of *Bathyplectes* spp. Comparatively few reports are available regarding direct toxicity of any sprayed Bt products on natural enemies of other insect pests (Johnson et al., 1995; Dutton et al., 2003; Schoenly et al., 2003; Chen et al., 2008; Dhillon and Sharma, 2010). Chen et al. (2008) did not show any significant difference in the parasitism rate of *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) in diamondback moth *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) larvae reared on *B. thuringiensis*-treated versus untreated broccoli leaves, under laboratory conditions. When rice fields were sprayed with *B. thuringiensis aizawai* or water, no significant differences were recorded between treatments in the abundance of predators and parasitoids throughout cropping season (Schoenly et al., 2003). In contrast, Dhillon and Sharma (2010) showed negative impacts on parasitoids through prolongation of the parasitoid developmental period, and reduction in the parasitism and adult emergence in testing the effects of *B. thuringiensis* var. *kurstaki* spray on *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae), a larval parasitoid of the pod borer, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) on several chickpea genotypes.

Although the possible mechanisms for less harmful effects of SDS-502 on *Bathyplectes* spp. parasitism rates are unknown, parasitoid behaviour, plant host quality and the BeetleGone application timing could have contributed to such effects. *Bathyplectes* spp. can parasitize all the larval stages of *H. postica*. One study reported that parasitoids developing on higher quality hosts are often less affected by *B. thuringiensis* toxins (Walker et al., 2007). Because SDS-502 was applied to younger larval stages of *H. postica* in our study, the preferred host stage of *H. postica* for parasitization could be less exposed to the Bt, and thereby the application timing could have further contributed to minimal harmful effects on parasitoid development. However, there was a tendency of lower parasitism at the higher rate of SDS-502 compared to the carrier control based on stem-cut sampling. Further laboratory and long-term field investigations are warranted to determine whether the direct and indirect exposure to SDS-502, particularly at the higher rate used in our study has any negative impact on the biology and population development of *Bathyplectes* spp.

Three *H. postica* strains including Eastern, Western and Egyptian are known to exist in the United States (Bundy et al., 2005). Specifically, the Western strain is known to occur predominantly in Northwestern region (e.g., Montana, Wyoming, Oregon, Idaho, Washington and Colorado); Western/Egyptian and Eastern/Egyptian strain alone or their mixtures are in Southwestern region (e.g., California and Arizona); and the Eastern strain in most of the Eastern region (e.g., Ohio New York and other states) of the United States (Bundy et al., 2005; Böttger et al., 2013). Despite numerous efforts to release several parasitoid species in 1990s, *B. curculionis* is the only species known to establish very well and it has been effective weevil parasitoid in the Western region of the United States (Ayedh et al., 1996; Brewer et al., 1997; Rand, 2013). In contrast, *B. anurus* and *M. aethiopoides* are two dominant parasitoid species contributing significantly to *H. postica* control in the Eastern region of United States (Kingsley et al., 1993; Oloumi-Sadeghi et al., 1993). Factors such as climatic conditions (Day, 1981), differential encapsulation of parasitoid eggs by *H. postica* strains (Maund and Hsiao, 1991) and parasitoid synchrony with peak host densities (Dowell and Horn, 1977) were reported as influencing the regional differences in parasitoid establishment and spread. Therefore, more studies on the performance of SDS-502 against the Egyptian strain and/or Eastern/Western strain mixtures and their compatibility with other parasitoids are necessary to understand and improve overall control efficacy of *H. postica*.

In summary, our results indicate that *B. thuringiensis* SDS-502 could be used for the control of *H. postica* in Montana. Because we found minor harmful impacts of the Bt to the larval parasitoids of *H. postica*, this product has a potential for incorporation into an *H. postica* integrated pest management program.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jip.2018.02.006.

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