Journal of Entomology and Nematology Vol. 4(5), pp. 49-53, August 2012
Available online at http://www.academicjournals.org/jen
DOI: 10.5897/JEN12.006
ISSN 2006- 9855 ©2012 Academic Journals

Full Length Research Paper

Virulence of *Beauveria bassiana* against Sunn pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) at different time periods of application

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Accepted 7 August, 2012

The virulence of *Beauveria bassiana* to overwintering Sunn pest adults was determined in the laboratory using micro-application techniques. Five microliter of 2×10^3, 2×10^4, 2×10^5, 2×10^6, 1×10^7, 2×10^7, 1×10^8 conidia/ml^{-1} were used in November, January and March. Applications were made to the mesosternum of adults. Mortality was recorded every 2 days for 16 days. Isolates were efficacious to Sunn pest at the higher concentrations. The cumulative corrected mortality after 12 days varied from 2.3% (SPT22) at 2×10^3 conidia/ml^{-1}, when treatment was conducted in November, to 100% (SPSR2) at 1×10^8 conidia/ml^{-1} in March. The LC_{50} for SPSR2 decreased from 1.1×10^7 to 3.7×10^3 conidia/ ml^{-1} when treatment was conducted in November and March, respectively. The 50% lethal time (LT_{50}) varied from 17.07 days for GHA (the commercial fungal isolate – in *BotaniGard*), to 9.31 days for SPSR2 in November. The most efficacious isolate was SPSR2.

Key words: Sunn pest, *Eurygaster integriceps, Beauveria bassiana*, LC_{50}, LT_{50}.

INTRODUCTION

Sunn pest, *Eurygaster integriceps* Puton, is a major pest of wheat in Eastern Europe and the near and Middle East (Parker et al., 2011). The vegetative stage of wheat and maturing grains are affected by adults and nymphs. Sunn pest feeds on leaves, stems and grains (Hariri et al., 2000). Through feeding on the grain, the insects inject a prolyl endoprotease (Darkoh et al., 2010). This enzyme causes extensive breakdown of gluten, which greatly reduces the baking quality of the dough (Hariri et al., 2000).

Control of Sunn pest mostly relies on the use of chemical insecticides; around US$ 40 million is spent each year on chemical pesticides (El-Bouhssini et al., 2009). The continuous use of chemical insecticides for control has resulted in serious management problems such as resistance to the insecticides, pest resurgence, elimination of beneficial insects and toxicity to humans and wildlife (Hendrawan and Ibrahim, 2006). These problems and the requirement for pesticide-free foods have increased pressure to find alternative management strategies (Mahdneshin et al., 2009). Integrated pest management (IPM), which utilizes ecological factors such as parasitoids, predators and microbial control agents are very attractive alternatives to the conventional chemicals used in the management of plant pests and diseases (Hanh et al., 2007). Entomopathogenic fungi that parasitize insects are valuable weapons for biocontrol and play an important role in promoting IPM (Cooke, 1977). *Beauveria bassiana* (Blasamo) Vullemin is the most prevalent fungus attacking *E. integriceps* populations in countries where this insect is a problem (Parker et al., 2003). Various strains of *B. bassiana* have been studied and have shown potential for inclusion in an IPM

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Table 1. Isolate designation, source and country of origin of *B. bassiana* isolates.

Isolate	Host	Country of origin
GHA	*Bemisia* sp.	USA
SP22	Litter	Turkey
SP566	Sunn pest	Iran
SPSR2	Sunn pest	Syria

program for Sunn pest (Edgington et al., 2007). The objective of the research herein was to determine the virulence of some *B. bassiana* isolates collected from different regions against overwintering Sunn pest under laboratory conditions.

MATERIALS AND METHODS

Sunn pest adults were collected from overwintering sites under the litter around pine trees from the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria at three different time periods in early and late winter and spring (November, 2008; January and March, 2009). In each date, Sunn pest was collected one day before treatment and stored in plastic ventilated containers in a refrigerator at 5°C until used. Four Isolates of *B. bassiana,* SP22, GHA (the active fungal ingredient of commercially available *BotaniGard*), SP566 and SPSR2 were obtained from the fungal culture collection maintained at ICARDA; isolate designation, source and country of origin of initial isolates are given in Table 1.

For each of the treatments, five culture plates were prepared by spreading 100 µl of 1×10^6 conidia/ ml^{-1} onto quarter strength Sabouraud dextrose agar supplemented with 0.25% technical yeast extract (SDAY/4- neopeptone 2.5 g/L, dextrose 10 g/L, agar 15 g/liter, and yeast 2.5 g/L; all Difco, Becton-Dickinson) (Parker et al., 2003). Plates were held for 14 days at 22 ± 2°C and 65 ± 5% rH, to maximize spore production. Conidia were then harvested by flooding each plate with 10 ml^{-1} of sterile distilled water (SDW) containing 0.01% (v/v) Tween 80 (Sigma) (Liu et al., 2003) and dislodging the conidia into suspension by stirring with a glass rod. All samples were vortexed 3 min to break up the conidial chains or clumps. Conidia were separated from hyphae and substrate materials by filtration of the suspension through two layers of cheese-cloth. The concentrations of fungal conidia in suspensions were determined using a haemocytometer. Viability of conidia was determined by spreading conidial suspensions onto SDAY/4 in Petri dishes and incubated at 25 ± 2°C. The percentage of germinated conidia was quantified after incubation for 24 h by examining a minimum of 200 conidia from each of three replicate plates. Conidia with germ tubes equal to at least half the conidial length were considered germinated. Suspensions with >90% germination were used for treatments.

Adults were treated with *B. bassiana* isolates at 2×10^5, 2×10^6, 1×10^7, 2×10^7, 1×10^8 conidia/ ml^{-1}, and were repeated at the three time periods for adults collection. Concentrations were prepared in SDW containing Tween 80 (0.01% v/v). Ten overwintered adults (5 ♂ and 5 ♀) per isolate and concentration were used. To secure the insects for topical application individuals were placed on a strip of scotch tape dorsal side down, and 5 µl of conidial suspension applied/insect to the mesosternum. Control insects were treated with SDW containing Tween.

After the application was dry (20 min) insects were transferred to wheat growing in pots (7 cm diameter and 8.5 cm height) surrounded by clear plastic cages and incubated at 22 ± 2°C and 65 ± 5% rH for 14 days. A split-plot design with five replicates was used. Mortality was counted 4, 6, 8, 10 and 12 days post application. Sunn pest adults were considered dead if they failed to move following slight probing. Dead insects from each treatment were surface sterilized and kept separately in Petri dishes containing sterile paper toweling moistened with 0.10% streptomycin sulfate and 0.02% penicillin G (Lacey and Brooks, 1997). Dishes were then incubated at 22 ± 2°C and 65 ± 5% rH for 2 weeks to observe fungal outgrowth.

Statistical analyses

Cumulative mortality was corrected for natural mortality using Abbott's formula (Abbott, 1925), and normalized using an arcsine transformation, and then analyzed statistically using ANOVA. Means were separated using Fisher's Unprotected LSD at P= 0.05. The computations were done using GenStat Ed:10 (Payne et al., 2007). Probit analysis was used to estimate LC_{50} of the isolates with 95% confidence limits (CL) and LT_{50} values (SPSS, 1999).

RESULTS

Germination varied from 90 to 98% (unpublished data). Data for percentage corrected mortality of Sunn pest caused by four isolates of *B. bassiana* at different concentrations during three time periods of application are presented in Figure 1. Sunn pest mortality varied significantly depending on isolate (df=3, 36; F= 70.23; P<0.001), conidial concentration tested (df=24, 288; F= 26.56; P<0.001) and time period of application (df=2, 12; F= 209.62; P<0.001). Mortality of Sunn pest began ~5 days after application, and then increased slowly. It is clear that the increase in percent mortality was pronounced between the first and third time period of application for all isolates (df=6, 36; F= 16.58; P<0.001). Differences in mortality between the low conidial concentrations (2×10^3, 2×10^4 and 2×10^5 conidia/ml^{-1}) were not statistically significant for any of the isolates tested when treatments were made in November and January, but mortality was significantly different in March (df=6, 81; F= 44.66; P<0.001), between all concentrations. Moreover, significant differences (df= 48, 288; F=1.64; P= 0.007) were observed between the different concentrations, especially for high concentrations (2×10^6, 1×10^7, 2×10^7, 1×10^8 conidia/ml^{-1}), for all isolates at the three time periods of application. The mortality caused by SPSR2 was higher than the other isolates tested with a maximum Sunn pest mortality of 100% at 10^8 and 2×10^7 conidia/ml^{-1} when Sunn pest were treated in March. Whereas, the mortality was around 64 and 36% when Sunn pest were treated at the same concentrations, respectively, in November. When adults were treated with SP566, mortality was 100 and 90% with 10^8 and 2×10^7 conidia/ ml^{-1}, respectively, in March. While, mortality was around 49 and 30% at the same concentrations, respectively, when treatment was made in November. Mortality of SP22 was 38, 42 and 80% with 10^8 conidia/ml^{-1}, when Sunn pest was treated in November, January and March,

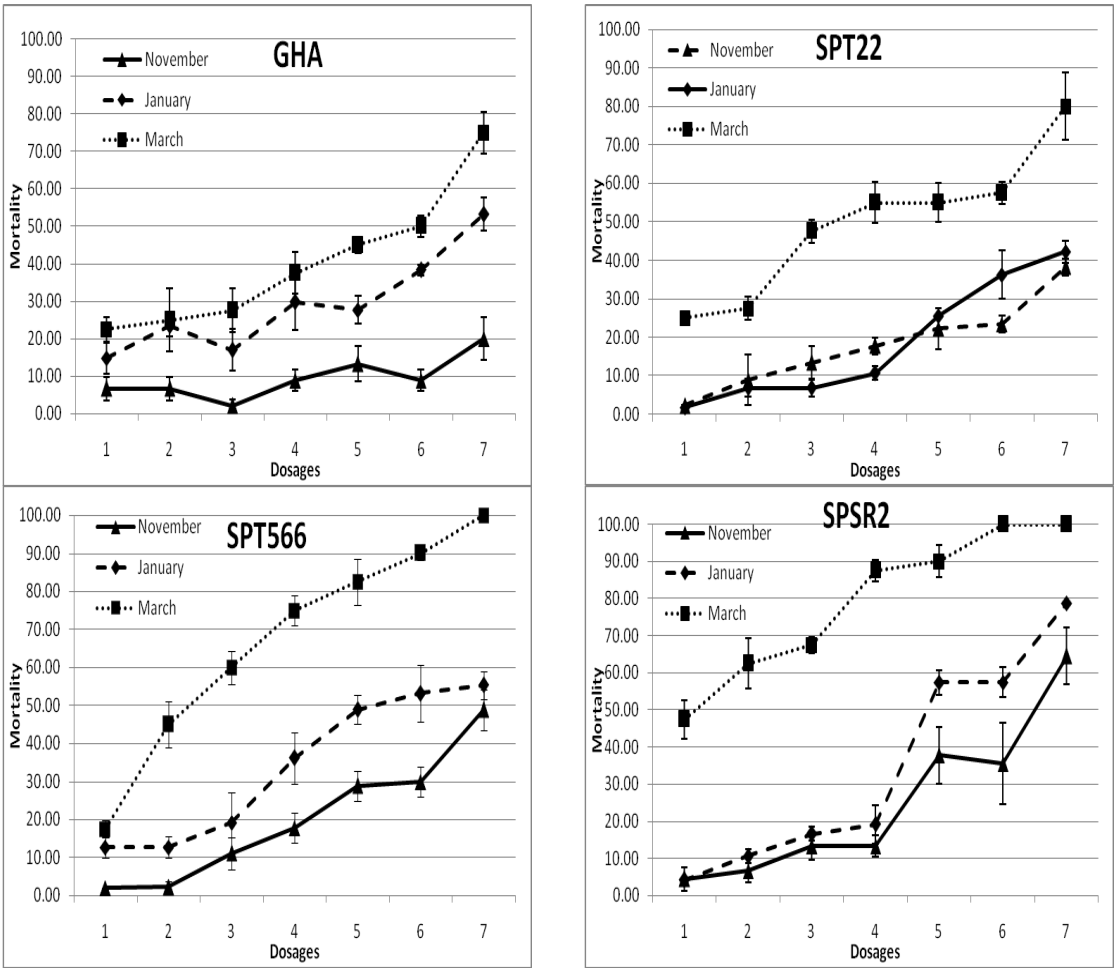

Figure 1. Percentage corrected mortality of Sunn pest caused by four isolates of *B. bassiana* at different concentrations during three time periods of application.

respectively. The lowest mortality was observed with GHA.

The LC_{50} estimates ranged from 3.7×10^{3} to 1.5×10^{13} conidia/ ml^{-1}, depending on isolates and time periods of application (Table 2). Sunn pest were more sensitive to infection by *B. bassiana* when treatment was made in March (time of Sunn pest migration to wheat fields). The lowest LC_{50} was 3.7×10^{3} (2.0×10^{3}- 1.1×10^{4}), conidia/ ml^{-1}, when Sunn pest were treated in March with SPSR2, compared with 1.1×10^{7} (6.9×10^{5}- 2.1×10^{7}) conidia/ ml^{-1}, when they were treated in November. The highest LC_{50} 1.5×10^{13} (2.4×10^{10} - 8.2×10^{18}) was observed with GHA in November.

The LT_{50} values varied from 5.48 to 30.02 days Depending on isolate, concentration, and time of application (Table 3). The shortest LT_{50} (5.48 days) was obtained with SPSR2 at 10^{8} conidia/ml^{-1} in March. The highest LT_{50} (30.02 days) was obtained with GHA at 10^{7} conidia/ml^{-1} in November. Sunn pest was killed faster when treatment was made in March for all isolates at the three concentrations tested.

DISCUSSION

Although all isolates caused mortality to Sunn pest adults, they showed different levels of virulence. These findings corroborate previous studies that isolate-specific differences in virulence toward a single species of insect occurs (Haji Allahverdi Pour et al., 2008; Tsuda et al., 1996; Todorova et al., 1994). Under the same experimental conditions, the same insect host can be resistant to certain isolates of *B. bassiana*, while being susceptible to other isolates of the same pathogen (Tanada and Kaya, 1993; Todorova et al., 2002).

SPSR2 had significantly higher mortality (100%), and the lowest LT_{50} (Table 3) to Sunn pest adults than the other isolates, while GHA had the lowest mortality (75%) at the highest concentration (1×10^{8} conidia/ml^{-1}) when treatment was made in March. This is in agreement with Tanada and Kaya (1993) that isolates from the same hosts cause higher mortality to that host than those from other hosts. However, Haji Allahverdi Pour et al. (2008) showed that soil isolates had higher virulence to Sunn

Table 2. LC_{50} (conidia ml^{-1}) values with 95 fiducial limits and probit analysis parameters for adult Sunn pest at different time periods of application.

Isolates	Time periods of application	LC_{50} (conidia/ ml^{-1})	95% fiducial limits		Intercept (a)	Slope (b)	x^2 value	p-value
			Lower	Upper				
GHA	November	1.5×10^{13}	2.4×10^{10}	8.2×10^{18}	3.139	0.141	0.89	0.989
	January	2.0×10^{8}	4.6×10^{7}	1.0×10^{9}	2.787	0.266	0.67	0.995
	March	7.9×10^{6}	3.6×10^{6}	2.6×10^{7}	3.177	0.264	0. 73	0.994
SP22	November	6.7×10^{7}	3.2×10^{7}	2.2×10^{8}	1.848	0.403	0.32	1
	January	4.4×10^{7}	2.8×10^{7}	7.8×10^{7}	0.647	0.551	0.2	1
	March	9.02×10^{5}	4.2×10^{5}	2.1×10^{6}	3.301	0.285	0.7	0.995
SP566	November	4.8×10^{7}	2.5×10^{7}	1.3×10^{8}	1.685	0.431	0.25	1
	January	1.8×10^{7}	7.6×10^{6}	5.0×10^{7}	2.843	0.297	0.61	0.996
	March	7.8×10^{4}	4.1×10^{4}	1.2×10^{5}	2.544	0.502	0.34	0.999
SPSR2	November	1.1×10^{7}	6.9×10^{6}	2.1×10^{7}	1.621	0.48	0.25	1
	January	3.5×10^{6}	2.9×10^{6}	5.5×10^{6}	0.746	0.116	0.18	1
	March	3.7×10^{3}	2.0×10^{3}	1.1×10^{4}	3.665	0.375	0.28	0.977

Table 3. LT_{50} values in days for Sunn pest adults treated with different isolates of *B. bassiana* at 1×10^{7}, 2×10^{7} and 1×10^{8} conidia ml^{-1} at different time periods of application.

Isolate	Time period of application	Concentration		
		1×10^{7}	2×10^{7}	1×10^{8}
		LT_{50} (days)		
GHA	November	30.02	17.68	17.07
	January	15.38	15.05	11.77
	March	11.92	11.68	9.95
SP22	November	16.40	14.88	10.75
	January	14.29	13.83	11.27
	March	9.31	9.52	8.22
SP566	November	15.08	13.16	11.78
	January	11.44	11.98	10.13
	March	7.91	7.41	5.22
SPSR2	November	13.29	12.12	9.31
	January	8.54	8.25	7.29
	March	7.12	6.34	5.48

pest adults than the isolates from the insect source.

Results of mortality showed that Sunn pest was more susceptible to infection by *B*. *bassiana* toward the end of the overwintering period (March), where percent mortality reached 100%. Sunn pest adults do not feed during the overwintering period, but food reserves are depleted and thus may increase the susceptibility from January to March. Kouassi et al. (2003) revealed that development of fungal pathogens within hosts can be influenced indirectly by host food. Furthermore, Hajek and St. Leger (1994) demonstrated that successful development of *B*. *bassiana* within hosts is based on overcoming the host hemocyte response.

The differences observed in LC_{50} estimates with the four different isolates tested could reflect genetic, physiological differences between isolates or factors such as toxins or characteristics of the host (Butt et al., 1992; Todoraova et al., 2002). Our experiments were conducted

using adults collected from overwintering sites. Because the adults spend about 9 months under the litter in these sites (Parker et al., 2011), we believe that these insects would be more vulnerable to fungi than adults in the field (summer populations). This may explain why Haji Allahverdi Pour et al. (2008) demonstrated that field-collected nymphs were more susceptible to infection by *B. bassiana* than new generation adults (summer populations). The variations observed between LC50's can be explained by variations in the virulence of a single isolate to related species of the host insect (Sabbahi et al., 2008).

Results of LT_{50} indicated that SPSR2 is more effective than the other isolates; this isolate killed Sunn pest faster than SP22 as reported by Parker et al. (2003). The increase of insecticidal activity of conidia against Sunn pest through application time periods (as referenced above) may be related to the reduction of fat body in insects through the overwintering period.

In conclusion, adult Sunn pest are susceptible to some *B. bassiana* isolates. The most promising of these isolates was SPSR2, isolated from Sunn pest adults in wheat fields in the summer. This isolate should be tested further in an IPM program for Sunn pest.

REFERENCES

Abbott WS (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265-267.

Butt TM, Barrisever M, Drummond J, Schuler TH, Tillemans FT, Wilding N (1992). Pathoginicity of the entomopathogenus Hyphomycetes fungus *Metarhizum anisopliae* against the chrysomelid beetles Psylliodes chrysocephata and Phaedon cochleariae. Biocontrol. Sci. Technol. 2:327-334.

Cooke R (1977). The biology of symbiotic fungi. John Willey and Sons, New York.

Darkoh C, EL Bouhssini M, Baum M, Clack B (2010). Characterization of a prolyl endoprotease from *Eurygaster integriceps* Puton (Sunn Pest) Infested Wheat. Arch. Insect. Biochem. 74(3):163-178.

Edgington S, Moore D, EL-Bouhssini M, Sayyadi Z (2007). *Beauveria bassiana* for the control of Sunn pest (*Eurygaster integriceps*) (Hemiptera: Scutelleridae) and aspects of the insects daily activity relevant to a mycoinsecticide. Biocontrol. Sci. Technol. 17:63-79.

EL Bouhssini M, Street K, Joubi A, Ibrahim Z, Rihawi F (2009). Sources of Wheat resistance to Sunn pest, *Eurygaster integriceps* Puton, in Syria. Genet. Resour. Crop. Eviron. 56:1065-1069.

Hajek AE, St. Leger RJ (1994). Interaction between fungal pathogens and insect host. Annu. Rev. Entomol. 39:293-322.

Haji Allahverdi Pour H, Ghazavi M, Kharazi-Pakdel A (2008). Comparison of the virulence of some Iranian isolates of *Beauveria bassiana* to *Eurygaster integriceps* (Hem.: Scutelleridae) and production of the selected isolate. J. Entomol. Soc. Iran. 28:13-26.

Hanh Vu VIL, Hong S, Kim K (2007). Selection of Entomopathogenic Fungi for Aphid control. J. Biosci. Bioeng. 104:498-505.

Hariri G, Williams PC, Jaby EL-Haramein F (2000). Influence of Pentatomidae insects on the physical dough properties and two-layered flat-bread baking quality of Syrian wheat. J. Cereal Sci. 31:111-118.

Hendrawan S, Ibrahim Y (2006). Effect of dust formulations of three entomopathogenic fungal isolates against *Stiphilus oryzae* (Coleoptera: Curculionidae) in rice grain. J. Biosci. 17:1-7.

Kouassi M, Coderre D, Todorova SI (2003). Effect of plant type on the persistence of *Beauveria bassiana*. Biocontrol. Sci. Technol. 13:415-427.

Lacey LA, Brooks MW (1997). Initial handling and diagnosis of diseased insects. Pages 1-15. In: Manual of Techniques in Insect Pathology. L.A. Lacey (Editor). Academic Press, New York p. 409.

Liu H, Skiner M, Parker BL (2003). Bioassay method for assessing the virulence of *Beauveria bassiana* against tarnished plant bug, *Lygus lineolaris* (Him., Miridae). J. Appl. Entomol. 127:299-304.

Mahdneshin Z, Safaralizadah MH, Ghosta Y (2009). Study on the efficacy of Iranian isolates *Beauveria bassiana* (Blasamo) Vullemin and *Metarhizum anisopliae* (Metsch.) Sorokin against *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae). J. Biol. Sci. 9:170-174.

Parker BL, Amir-Maafi M, Skinner M, Kim J, EL-Bouhssini M (2011). Distribution of Sunn Pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae), in overwintering sites. J. Asia-Pacific Entomol. 14:83-88.

Parker BL, Skinner M, Costa SD, Goulli S, Reid W, EL Bouhssini M (2003). Entomopathogenic fungi of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae): collection and characterization for development. Biol. Control 27:260-272.

Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM (2007). GenStat for Windows (10th Edition) Introduction. VSN International, Hemel Hempstead.

Sabbahi R, Merzouoki A, Guertin C (2008). Efficacy of *Beauveria bassiana* (Bals.) Vuill. Against the tarnished plant bug, *Lygus lineolaris* L. in strawberries. J. Appl. Entomol. 132:124-134.

SPSS (1999). SPSS for Windows user's guide release 10. 1st Edn. SPSS inc., Chicago.

Tanada Y, Kaya HK (1993). Insect pathology. Academic Press. New York, p. 665.

Todorova SI, Cloutier C, Cote JC, Coderre D (2002). Pathogenicity of six isolates of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina, Hyphomycetes) to *Perillus bioculatus* (F) (Hem., Pentatomidae). J. Appl. Entomol. 126:182-185.

Todorova SI, Cote JC, Martel P, Coderre D (1994). Heterogeneity of two *Beaveria bassiana* strains revealed by biochemical test. Protein profiles and bio-assays on *Leptinotarsa decemlineata* (Col., Chrysomelidae) and *Coleomegilla maculata lengi* (Col., Chrysomelidae). J. Appl. Entomol. 120:159-163.

Tsuda K, Yoshioka T, Tsutsumi T, Yamanaka M, Kawarabata T (1996). Pathogenicity of some entomogenous fungi to brown-winged green bug, *Plautia stali* (Hemiptera: Pentatomidae). Jpn. J. Appl. Entomol. Z. 40:318-321.