Bulletin of Insectology **65** (2): 161-170, 2012
ISSN 1721-8861

Adaptation of the invasive western conifer seed bug *Leptoglossus occidentalis* to Trentino, an alpine region (Italy)

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Abstract

Non-native organisms can affect native communities and ecosystems in different ways. We examine here the case of the western conifer seed bug *Leptoglossus occidentalis* Heidemann (Heteroptera Coreidae), a polyphagous pest of conifer seeds, introduced from northern America into Italy in 1999 and then spreading across the whole Europe. The bug was detected in alpine forests of Trentino (northern Italy) in 2002, and since then known mainly as a nuisance agent for its habit to overwinter inside buildings. The lack of information on the ecology in mountain areas led us to investigate its distribution in some alpine pine stands, in relation to altitude and to fungal pathogens potentially associated. The presence of *L. occidentalis* was observed in all main geographic areas of Trentino, up to the subalpine belt in the southern part of the region. The seed bug completed one or two generations depending on altitude and local climatic conditions. Field data and rearing under artificial conditions indicated the importance of heat accumulation for the development of nymphal instars and the role of temperature thresholds in regulating adult behaviour. The seed bug was observed on trees (*Pinus nigra*, *P. sylvestris*) as well as on shrubs (*P. mugo*), facilitating the rapid colonisation of a fragmented mountain environment. This may have important implication in the dispersal of pathogens, as spores of *Diplodia pinea* were detected on adults. Although a direct economic impact has not been evidenced yet in this alpine area, an ecological impact hampering natural regeneration, especially in high-altitude forest ecosystems, can be envisaged.

Key words: ecology, spread, pine stands, *Diplodia pinea*, insect-pathogen association.

Introduction

Adventive (non-native) plant or animal species spread beyond their native range due to intentional or unintentional means. If natural dispersal may sometimes occur, in most of the cases human activities are responsible for unintentional introductions. When an organism colonizes a new area, it can be integrated into novel communities (Sailer, 1978) or it can affect them (Richardson, 2005): species that may impact the new ecosystems and cause several problems become invasive.

Alien terrestrial invertebrates represent one of the most numerous groups of introduced organisms in Europe. Among them, arthropods, mostly insects, are nearly the 94% (Roques *et al*., 2009). Italy is the European country with the highest number of alien arthropod species because of its climatic parameters, which allow the establishment of both subtropical and northern species, and the position in the middle of Mediterranean basin, which makes it a commercial and tourist crossroad (Pellizzari *et al*., 2005; Roques, 2010). Most of the alien insect species are Hemiptera (64%) and in some cases Italy represents the first focus for species that subsequently expand towards neighbouring countries (Pellizzari *et al*., 2005).

One of the insect species recently introduced in Europe is the western conifer seed bug, *Leptoglossus occidentalis* Heidemann (Heteroptera Coreideae), a polyphagous pest of coniferous trees (Hedlin *et al*., 1981; Koerber, 1963). It has been reported feeding on about fourteen American and European conifer species: *Pinus* spp., *Calocedrus decurrens* (Torr.) Florin, *Pseudotsuga menziesii* (Mirb.) Franco, *Tsuga canadensis* (L.) Carrière, *Cedrus* spp., *Abies* spp. and *Picea* spp. (Koerber, 1963; Krugman and Koerber, 1969; Villa *et al*., 2001).

First reported in the western North America, from the second half of 1900 the seed bug has expanded also to the eastern part of the United States and Canada (Bates, 2002; Cibrián-Tovar *et al*., 1986; Gall, 1992; Heidemann, 1910; Marshall, 1991; McPherson *et al*., 1990; Ridge-O'Connor, 2001; Schaffner, 1967; Scudder, 2008) and was accidentally introduced to Europe in 1999. The insect was first discovered in northern Italy, and soon spread to other parts of Italy (Maltese *et al*., 2008; Salvadori, 2004; Tescari, 2001; Villa *et al*., 2001). Subsequently, it was found in other European countries (Dusolier *et al*., 2007; Fent and Kment, 2011; Jurc and Jurc, 2005; Kment and Baňař, 2008; Lis *et al*., 2008; Malumphy *et al*., 2008). Recently, the seed bug was found for the first time in Japan (Ishikawa and Kikuhara, 2009).

The strong flight ability and the artificial transport of specimens (e.g. as hitchhiker or with tree shipments) are supposed to have strongly accelerated the spread of this species (Blatt, 1994; Dusolier *et al*., 2007; Gall, 1992; Malumphy *et al*., 2008; Ridge-O'Connor, 2001; Wheeler, 1992).

Although *L. occidentalis* was first considered as monovoltine in North America (Hedlin *et al*., 1981; Koerber, 1963), recent observations suggest that a partial second generation may exist in Canada (Bates, 1999), whereas two to three generations occur annually in Mexico (Cibrián-Tovar *et al*., 1986). In northern Italy, *L. occidentalis* can accomplish two or three generations per year (Bernardinelli *et al*., 2006). In autumn *L. occidentalis* adults seek sheltered locations, in which they aggregate and overwinter (Koerber, 1963). Natural overwintering shelters have been identified in pine bark (Downes, 1927), dead, dry Douglas firs (Dennys, 1927) and in nests of hawks and rodents (Hussey, 1953).

As both adults and nymphs feed by inserting their stylets into cones and digesting the content of developing seeds, this species can cause serious economic losses in high-value seed orchards (Bates and Borden, 2005; Bates *et al*., 2002a; Strong *et al*., 2001). The seed bug has also been hypothesized to have a negative impact on the production of pine-seeds for human consumption in Italian stone pine (*Pinus pinea* L.) stands (Benassai *et al*., 2007; Roversi *et al*., 2011). In addition, the bug is often considered an urban pest, causing alarm and nuisance when lots of adults invade buildings for overwintering and damaging common plumbing materials (Bates, 2005; Blatt, 1994; Gall, 1992).

Moreover, the introduction of a new herbivore species in native forest ecosystems can influence the interactions with other organisms, namely fungal pathogens that are known to use insects as vectors (Kluth *et al*., 2002; Martin, 1992; Webber and Gibbs, 1989). Only few studies focused on the transmission of fungal diseases by cone and seed insects (Battisti *et al*., 1999; Feci *et al*., 2002; 2003; Haddow and Newman, 1942; Hoover *et al*., 1996). *Diplodia pinea* (Desm.) Kickx is a very common and widely spread conifer pathogen occurring on various species in the *Pinaceae* (Stanosz, 1997). Depending on climatic and environmental conditions, this fungus may exist in a latent asymptomatic phase, growing as saprotroph, or become pathogenic, causing a rapid death of currently expanding shoots (Stanosz *et al*., 2001; Maresi *et al*., 2002). Conidia are disseminated primarily by rain splash or air currents (Peterson, 1981). The association between the presence of Diplodia blight of pines and insect infestations was suggested by Zwolinski *et al*. (1994) and confirmed by Feci *et al*. (2003). More recently, a study conducted by Luchi *et al.* (2012) revealed that also *L. occidentalis* could be involved in the transmission of this fungal disease to *P. pinea*.

This work was aimed to a) describe the distribution of *L. occidentalis* in the different valleys of Trentino; b) study biological and ecological aspects, such as the voltinism, the development rate and the host preference, under different natural and artificial conditions; c) assess the association between the seed bug and *D. pinea*, the causal agent of Diplodia blight of pine.

Materials and methods

Surveys on the distribution

The presence of *L. occidentalis* in pine stands of Trentino (northern Italy) was investigated in September 2008. Autumn is a transition period in which adults can be observed on both pine trees and man-made structures. The following geographic areas (valleys) were considered: Alto Garda and Valle dei Laghi, Val di Cavedine, Val di Ledro, Val Giudicarie Inferiori, Val Rendena, Val di Sole, Val di Non, Valle dell'Adige, Vallagarina, Valsugana, Valle di Primiero, Valle del Vanoi, Val di Fiemme and Val di Fassa. As the main objective of these surveys was the assessment of the seed bug spread, inspections were limited to significant points of each valley and took in account the different site characteristics, such as the kind of vegetation, the presence of buildings and altitude. To increase the probability of finding this insect, observations were based on the map of the distribution of Trentino pine stands (figure 1). The survey was conducted by choosing randomly fifteen pine trees in each point. A visual inspection was conducted on the canopy of each tree, with the help of binoculars too, at midday. To standardise the investigations, the presence of the seed bug was assessed by an observation period limited to three minutes. The survey was extended to man-made structures close to the stands, integrating the data collected with interviews to forest personnel and local inhabitants.

Study area and climate

The study areas for biology and ecology are located in the Alto Garda area (southern Trentino, sites A in figure 1). Alto Garda is characterised by a transition climate between the Mediterranean and Alpine ones especially because of the thermal influx of the lake and high mountains (Keller *et al*., 1994).

Two low-altitude areas (A_1 and A_2) were selected. Area A_1 (45°59'16"N, 10°55'49"E) is located north of Dro town and west of Drena town at 220 m a.s.l., is south-west exposed and encompasses about 2000 m^2. Area A_2 (45°51'53"N, 10°53'40"E) is located over Torbole town and near Nago town in Daine place. The observation site is situated at 390 m a.s.l., with western exposure, and is about 5000 m^2 wide. The site shows no significant thermal differences with the bottom of the valley. Both areas are on calcareous bedrock. In the past, areas A_1 and A_2 were reforested with *Pinus nigra* Arnold, which represents the main species. In minority, also *Fraxinus ornus* L., *Ostrya carpinifolia* Scopoli, *Pinus sylvestris* L., *Quercus pubescens* Willdenow and *Cupressus sempervirens* L. are present. In the areas the pine stands are patchy, *P. nigra* being younger than the rest, about 4 m high, with a clustered or isolated distribution and with a well developed foliage. Unlike area A_2, area A_1 showed in the past severe attacks of the coelomycete *D. pinea.*

High-altitude surveys were conducted along the western side of Monte Baldo. Area A_3 is located near Malga Casina at 1040 m a.s.l. (45°50'40"N, 10°53'43"E), area A_4 in the locality Prati di Nago at 1420 m a.s.l. (45°50'03"N, 10°53'37"E) and area A_5 on the eastern side of Monte Varagna at 1730 m a.s.l. (45°49'33"N, 10°53'47"E). The survey area A_3 is about 700 m^2 wide, with a northern exposure and a slope of 17%; both zones A_4 and A_5 are around 900 m^2 wide, with a slope of 19% and face north-west and south-east, respectively. High-elevation areas comprise different vegetation belts. Austrian pine wood climbs up, with some discontinuities, from Torbole village (85 m a.s.l.) to Dos Casina (980 m a.s.l.), where an almost pure Scots pine wood takes over up to 1200 m a.s.l.. Area A_3 represents a terminal patch of this pinewood with sparse 4 m high *P. sylvestris* trees. A beech (*Fagus sylvatica* L.) wood, with a good presence of Norway spruce (*Picea abies* Karst.), follows. In area A_4 there are some isolated 7 m high *P. sylvestris* and *Larix decidua* Miller trees on a clearing pasture. In area A_5, which is beyond the tree line, the grass-rock slope is broken up by several dwarf mountain pine (*Pinus mugo* Turra) shrubs grouped with few isolated larch, Norway spruce and Scots pine trees.

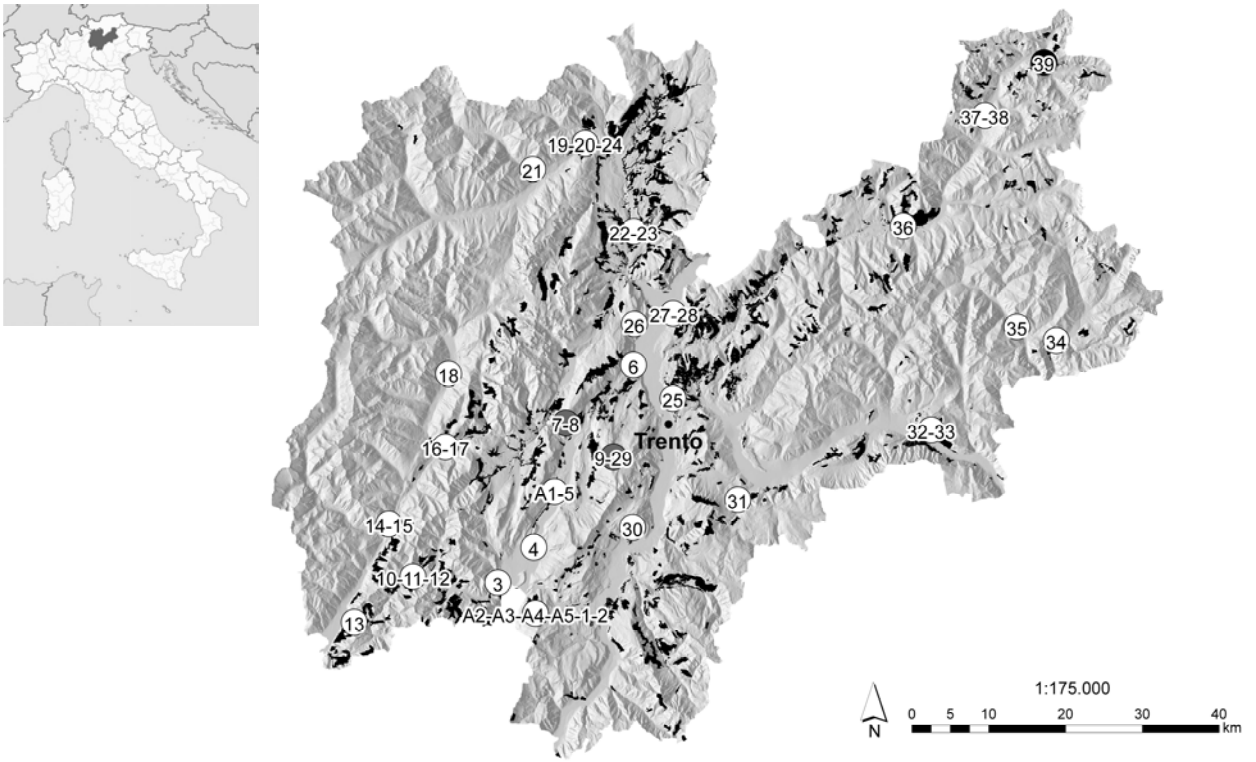

Figure 1. Distribution map of *L. occidentalis* in the main Trentino valleys. The chart shows the pine stands (in black) present in the region, the surveyed sites (numbers) and the study areas (letters). White circles represent the presence of the seed bug, dark ones the absence. Grey circles indicate the overlapping of white and dark circles (PAT Servizio Foreste e Fauna – Sistema Informativo Ambiente e Territorio).

Study of the voltinism

Field observations

The biology of *L. occidentalis* under natural conditions was studied by field observations between June 2007 and October 2009. Small-sized trees, up to about 4 m high, with a well developed canopy were chosen in each area. Twenty-five *P. nigra* were selected in area A_1 and A_2, ten *P. sylvestris* in area A_3, ten *P. sylvestris* and *L. decidua* in area A_4 and ten *P. mugo* in area A_5. Surveys were conducted about three times a month during the activity periods of the seed bug (under Trentino climatic conditions, from May to end of September-beginning of October). The canopy of each sampled tree was examined for three minutes, recording the number of individuals, the developmental instar, the localization of the seed bugs on plants (cones or needles), the activity carried out (e.g. feeding or resting) and air temperature.

As suggested by Bernardinelli *et al.* (2006), the Day Degree (DD) method based on heat accumulation may help in predicting the development rate and the number of generations of *L. occidentalis*. Considering the threshold value of 13.5 °C (proposed by Bernardinelli *et al.*, 2006), eggs hatch between 81 and 126 DD; the development from 1^{st} instar nymph to adult, fixed a threshold value of 14.6 °C, requires 287-460 DD. Following these indications and using the DD values provided by IASMA automatic meteorological station of Nago (near area A_2), it was possible to build up a phenological forecasting model. The field-observed and predicted data were compared to assess the efficacy of the model under the climatic conditions of Trentino.

Rearing under artificial conditions

To study the biological cycle under artificial conditions, a seed bug rearing was established in a building in the town of Arco (45°55'01"N, 10°53'01"E, 92 m a.s.l.), close to the natural sites, from July 2007 to August 2009.

Adults, collected manually from lower pine branches (maximum height 2.5 m) in the area A_2 between the end of July and the beginning of August, were transferred in plastic boxes (39.6 cm long, 13.2 cm high and 27.8 cm deep), covered with an insect-proof net. Fifteen adults (10 females and 5 males) were put in each box and fed with short branches bearing conelets (6-8 per box). Besides *P. nigra* and *P. sylvestris*, different other species were considered: *C. sempervirens*, *L. decidua*, *P. abies* and *P. mugo*. The branches were replaced with fresh material once a week. With temperatures higher than 25.0 °C, the humidity within the boxes was increased by a spray bottle. A total number of six boxes (one per tree species) were prepared and put under indoor conditions (unheated room facing north-east, with natural light and ventilation). The temperature varied during the year between 8.0 and 27.0 °C, with a daily temperature range lower than 3.0 °C and air humidity between 45 and 60%.

The insects were accurately controlled and observed every three days, recording the main behavioural and biological features, such as feeding, mating, egg laying and nymphal development.

Evaluation of the fungus-insect association

To assess the possible role of *L. occidentalis* as vector of *D. pinea*, the presence of the fungus conidia on the insect body was ascertained following Battisti *et al.* (1999) and Feci *et al.* (2002). In November 2007 seed bug adults were collected inside some buildings located in three different locations: 40 specimens in San Michele all'Adige (Valle dell'Adige, 46°11'35"N, 11°08'07"E, 218 m a.s.l., circle 27 in figure 1), 40 specimens in Lagolo (Val di Cavedine, 46°02'08"N, 11°00'08"E, 933 m a.s.l., circle 8 in figure 1) and 16 specimens in Dos Casina, near Nago-Torbole (Alto Garda, 45°50'40"N, 10°53'43"E, 1040 m a.s.l., circle A_3 in figure 1). The smaller number of specimens collected in the latter site is due to the lower population density. Insects were manipulated with forceps and gloves to avoid contaminations and stored in sterile BD Falcon™ 50 ml conical tubes at −20 °C. Each insect was washed in lab conditions in 1 ml sterile water with 1% detergent (Tween 80) by shaking for 1 min at 40 Hz. The resultant suspension was then recovered and concentrated in a stove at 40 °C to the volume of 100 µl.

Conidia presence was assessed by microscope observation (20× magnifications) of the whole suspension after staining with 10 µl methyl blue.

The differences between populations in the proportion of samples with *D. pinea* conidia were evaluated by the chi-squared (χ^2) test.

Table 1. List of locations where the bio-ecological observations on *L. occidentalis* were conducted (A_1-A_5) and its presence was investigated (1-39). Identification (N°), geographic coordinates, the type of wood, altitude and presence are reported for each location.

Geographic area	Location	N°	Coordinates	Site	Altitude (m)	Presence
Alto Garda and V. dei Laghi	Dro	A_1	45°59'16"N, 10°55'49"E	*P. nigra* stand	220	x
	Nago-Torbole	A_2	45°51'53"N, 10°53'40"E	*P. nigra* stand	390	x
	Nago-Torbole	A_3	45°50'40"N, 10°53'43"E	*P. sylvestris* stand	1040	x
	Nago-Torbole	A_4	45°50'03"N, 10°53'37"E	*P. sylvestris* stand	1420	x
	Nago-Torbole	A_5	45°49'33"N, 10°53'47"E	*P. mugo* stand	1730	x
	Nago-Torbole	1	45°51'50"N, 10°52'45"E	building	156	x
	Nago-Torbole	2	45°50'42"N, 10°53'42"E	building	1040	x
	Riva del Garda	3	45°53'03"N, 10°49'46"E	*P. nigra* stand	585	x
	Arco	4	45°55'28"N, 10°53'28"E	*Pinus* spp. (urban garden)	94	x
	Dro	5	45°59'24"N, 10°55'34"E	building	211	x
	Terlago	6	46°08'03"N, 11°03'59"E	*P. nigra* stand	801	x
	Ranzo	7	46°04'33"N, 10°57'20"E	*P. nigra* stand	1421	
	Ranzo	8	46°03'42"N, 10°56'39"E	building	702	x
Val di Cavedine	Lagolo	9	46°02'08"N, 11°00'08"E	building	933	x
Val di Ledro	Locca	10	45°54'01"N, 10°43'33"E	building	750	x
	Tiarno di Sotto	11	45°53'41"N, 10°41'05"E	building	753	x
	Tiarno di Sopra	12	45°53'20"N, 10°40'08"E	building	763	x
Val Giudicarie Inferiori	Storo	13	45°50'30"N, 10°35'14"E	*P. nigra* stand	412	x
	Agrone	14	45°57'25"N, 10°38'56"E	*P. nigra* stand	743	x
	Agrone	15	45°57'14"N, 10°38'55"E	building	637	x
	Preore	16	46°02'41"N, 10°44'48"E	*P. sylvestris* stand	639	x
	Preore	17	46°02'35"N, 10°44'45"E	building	555	x
Val Rendena	Caderzone	18	46°07'45"N, 10°45'16"E	building	754	x
Val di Sole	Tozzaga	19	46°23'02"N, 10°58'11"E	*P. nigra* stand	758	x
	Tozzaga	20	46°23'00"N, 10°58'18"E	building	697	x
	Arnago	21	46°21'55"N, 10°54'16"E	*P. nigra* stand	1084	x
Val di Non	Denno	22	46°16'35"N, 11°02'58"E	*P. nigra* stand	410	x
	Priò	23	46°18'12"N, 11°05'45"E	*P. nigra* stand	789	x
	Livo	24	46°24'16"N, 11°01'03"E	*P. nigra* (urban garden)	727	x
Valle dell'Adige	Martignano	25	46°05'38"N, 11°07'53"E	building	395	x
	Fai della Paganella	26	46°10'54"N, 11°04'11"E	building	971	x
	S. Michele a/A	27	46°11'35"N, 11°08'07"E	*P. nigra* stand	218	x
	S. Michele a/A	28	46°11'35"N, 11°08'07"E	building	218	x
	Viote del Bondone	29	46°01'10"N, 11°03'18"E	*P. mugo* and *P. abies* stand	1656	
Vallagarina	Pomarolo	30	45°56'41"N, 11°03'28"E	Mixed wood with *P. sylvestris*	780	x
Valsugana	Centa S. Nicolò	31	45°58'26"N, 11°14'09"E	building	769	x
	Grigno-Ospedaletto	32	46°02'54"N, 11°33'51"E	*P. sylvestris* stand	489	x
	Grigno-Ospedaletto	33	46°02'54"N, 11°33'51"E	building	489	x
Valle di Primiero	Masi di Imer	34	46°08'51"N, 11°46'47"E	*P. sylvestris* stand	783	x
Valle del Vanoi	Canal S. Bovo	35	46°09'54"N, 11°42'51"E	*P. sylvestris* stand	766	x
Val di Fiemme	Tesero	36	46°17'13"N, 11°31'41"E	*P. sylvestris* stand	961	x
Val di Fassa	Soraga	37	46°24'04"N, 11°40'01"E	*P. sylvestris* stand	1297	x
	S. Giovanni di Fassa	38	46°25'27"N, 11°40'40"E	*P. nigra* (urban garden)	1358	x
	Canazei	39	46°28'19"N, 11°46'31"E	*P. sylvestris* stand	1447	

Results

Surveys on the distribution

The observations conducted in September 2008 allowed describing the occurrence of *L. occidentalis* in Trentino (figure 1). As listed in table 1, the seed bug was found in 36 out of 39 surveyed sites, the few exceptions being high-altitude sites. Most of the detections on pine trees took place in the forest, but in some cases *L. occidentalis* was observed also in urban habitat. Individuals were more likely to be detected next to green cones. Among man-made structures, overwintering adults were found within woodpiles and wooden objects in unheated sheds. Moreover, insects were observed inside occupied buildings or into wooden shutters of deserted ones.

Study of the voltinism and bio-ecology

Field observations

The survey in natural conditions allowed finding the seed bug on a number of plant species, including hosts different from pines. The number and duration of generations were established at different altitudes (areas A_1-A_2 and A_3) and in two consecutive years. In this study, the generations were singled out by the comparison of rearing conditions with the population development observed in the field. As shown in figure 2, the number of generations is dependent on the elevation. *L. occidentalis* can complete one generation in area A_3 (1040 m) and two generations in areas A_1 and A_2 (220 and 390 m, respectively). The phenology is dependent on the weather conditions which may vary between years: in 2009 the overall development rate from adult to the next generation adult (Δ) was shortened due to higher mean temperatures resulting in a faster accumulation of degree days, compared to 2008. In area A_3, Δ was 90 days in 2008 and just 48 days in 2009. As well, in areas A_1 and A_2 Δ values of 76 and 53 days for the first generation and of 51 and 51 days for the second were recorded.

Based upon the DD method, a forecasting model for each generation of *L. occidentalis* in area A_1 and A_2 was

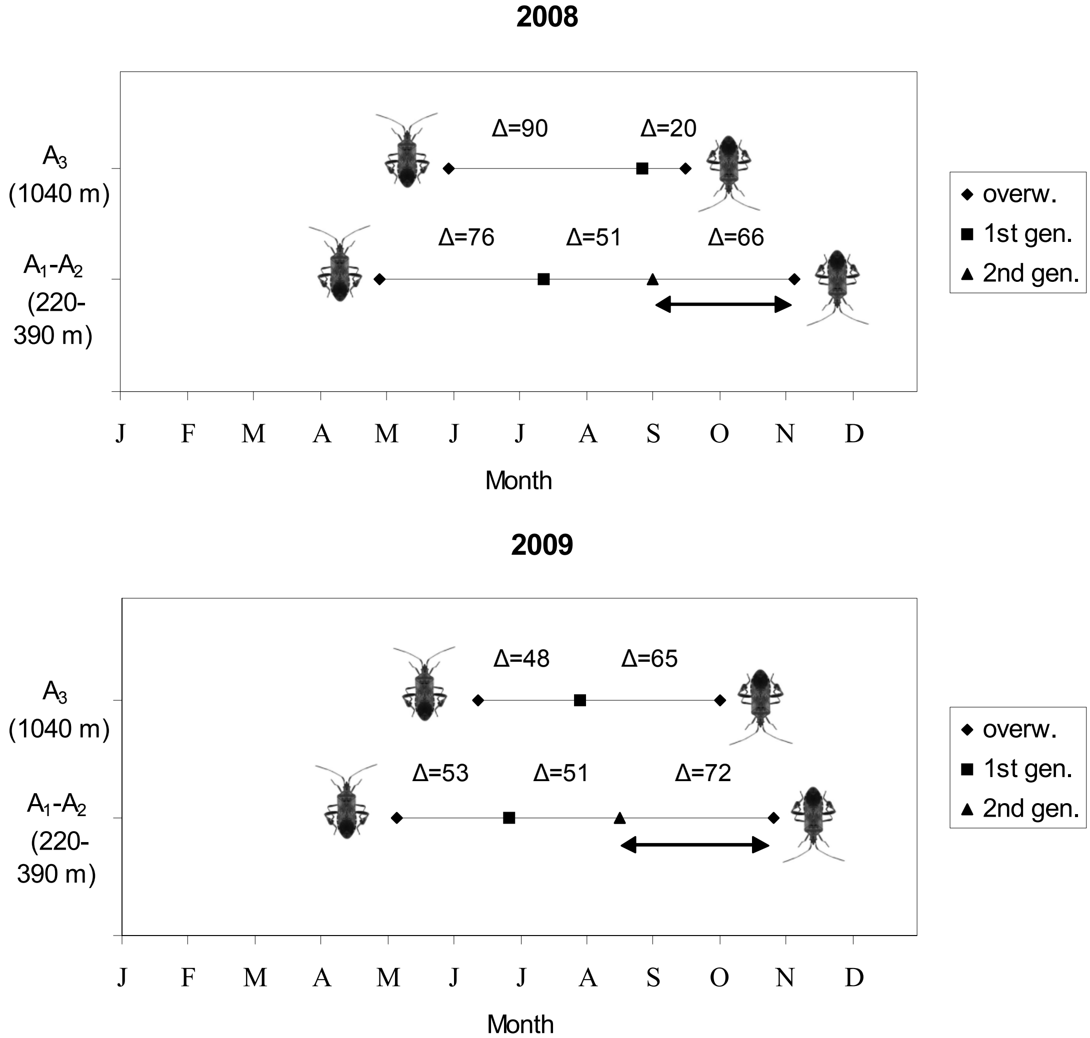

Figure 2. Scheme of *L. occidentalis* development recorded in 2008 (above) and 2009 (below) in the surveyed areas (A_1-A_2 and A_3). The emergence of the first adult from overwintering (overw. ↑), the emergence of first (1st gen.) and second (2nd gen.) generation adults on pines, the departure to overwintering sites (overw. ↓) as well as the interval between generations (Δ, in days) are showed. Double arrow (↔) indicates the presence of a partial third generation (nymphs that do not complete the development before winter).

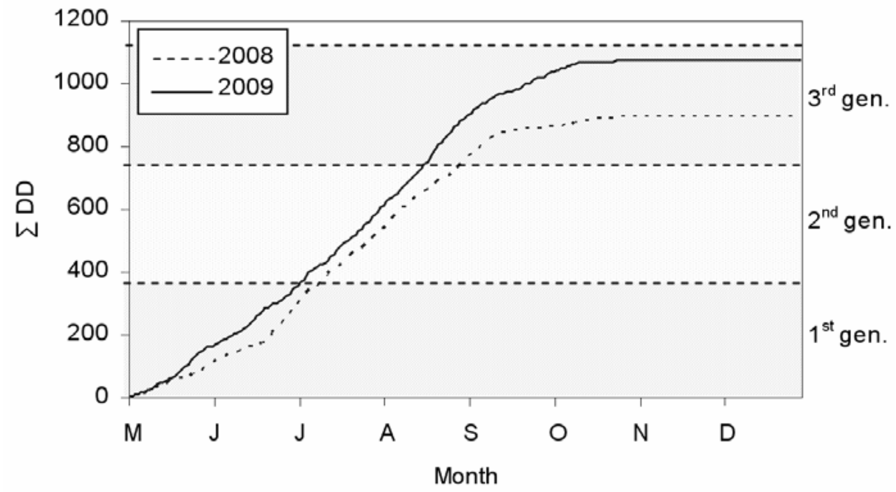

Figure 3. Cumulative Day Degree (DD) sum for the bottom valley study areas (A_1 and A_2) obtained integrating the DD calculated considering the threshold temperatures for egg and nymph development. Even though in 2009 heat accumulation was higher than in 2008, third generation nymphs did not develop into adults.

built up. The expected Δ values were 71 and 50 days in 2008 and 64 and 44 days in 2009, respectively.

The graph of the cumulative DD, obtained by integration of the DD calculated considering the two respective thresholds, shows the number of generations completed in 2008 and 2009. Even though climatic conditions in 2009 were more favourable than in 2008 for the development, the third generation was not completed before winter (figure 3).

In areas A_1, A_2 and A_3 *L. occidentalis* was regularly observed feeding on *P. nigra* and *P. sylvestris*, especially on conelets and needles (appendix A, B, C).

In the remaining survey areas (A_4 and A_5), the presence of the seed bug on *P. sylvestris* and *P. mugo* was confirmed, but the low population density did not allow following the development of generations. Besides *P. nigra* and *P. sylvestris*, eggs were also detected on *P. mugo* (appendix F).

L. occidentalis was noticed feeding on *L. decidua* only once, in area A_4 (appendix D).

The surveys on *L. occidentalis* in fall-winter season gave some indications about overwintering behaviour. Only adult insects, usually aggregated, were found close to man-made structures overlooking forests, especially in the south-facing sides.

Rearing under artificial conditions

Behavioural observations allowed identifying thermal ranges at which the seed bug becomes active in spring. In particular, 12-14 °C seems to be the threshold for starting/ending the activity, while between 14 and 18 °C a gradual increase of mobility and feeding activity was noticed. In the rearing site, *L. occidentalis* completed two generations in 2008. Considering *P. nigra*, eggs laid during July and August hatched about 12 ± 0.50 days (mean value ± S.E.) after oviposition. The mean nymphal development rate observed for the 1^{st} generation on this species was 32.75 ± 1.03 days, while for the 2^{nd} was 50 days. The Δ values showed by the seed bug under artificial conditions (1^{st} generation: 71 days; 2^{nd} generation: 50 days) were similar to those observed in areas A_1 and A_2 (1^{st} generation: 76 days; 2^{nd} generation: 51 days).

In artificial conditions the seed bug was confirmed feeding on *C. sempervirens*, *L. decidua*, *P. abies*, *P. mugo*, *P. nigra* and *P. sylvestris* (appendix E). Feeding activity was noticed on conelets, needles, small branches, buds, sporophylla and opened cones (appendix I).

Evaluation of the fungus-insect association

From each of the 96 specimens, 3 replications were obtained and a total number of 288 slides were examined. Positive samples contained at least one conidium with the typical morphological and dimensional characteristics of *D. pinea* (25-36 × 13-16 µm ellipsoidal to long ovoid spores, warty and generally dark brown) (appendix J). Seventeen positive specimens were found in S. Michele (42.5%), 14 in Lagolo (35%) and 6 in Dos Casina (37.5%). The main part of specimens showed no more than 1-2 conidia, even though in few cases up to 26 spores were observed. Similar percentages of positive samples were detected in the three populations ($\chi^2_{(5)} = 0.488$; $p = 0.993$).

Discussion

In this paper we showed that the western seed bug has a great capacity to adapt to very different conditions of climate and host plants, as those offered by a highly variable mountain habitat across an altitudinal gradient. The voltinism is the major trait affected as the insect can shift from one to almost three generations per year along a short, but steep, mountain slope. This may have a number of implications for the population growth, as we found that the insect overwinters only as an adult, which was reported also by Koerber (1963) and Strong (2006), and often the individuals have not reached this stage at the end of the summer. We hypothesize that the fluctuations of the populations observed for this species in the study area can be at least partly explained by the mismatch possibly occurring in some years, as observed in our two-year study.

L. occidentalis, detected for the first time in Torbole sul Garda (southern Trentino) in fall 2002, rapidly spread across Trentino covering all the main valleys already in 2008. In Alto Garda and Valle dei Laghi the seed bug was found out in pine stands from the bottom valley up to the tree line. Also in other wide valleys, with a good distribution of *P. nigra* and *P. sylvestris* (Valle dell'Adige, Val Giudicarie, Valsugana, Val di Non, Val di Sole), the detection of *L. occidentalis* was easy. On the other hand, a more difficult observation was noticed in closer valleys at higher altitude (Valle di Primiero, Val di Fiemme and especially Val di Fassa).

Extrinsic and intrinsic factors have been considered to explain *L. occidentalis* spread. Among them, the attitude of this species to spend a part of the life cycle within man-made structures enhances the role of human-mediated dispersal, through commercial or touristic trade, as already demonstrated by Dusolier *et al.* (2007), Gall (1992) and Malumphy *et al.* (2008). Moreover, studies conducted in northern America and Europe attribute to *L. occidentalis* good flight ability (Koerber, 1963; Gall, 1992; Malumphy *et al*., 2008). Finally, this insect was demonstrated able to utilize several species of American native and introduced conifers as food plants (Gall, 1992). Field observations and rearing experiments conducted in our research confirm the polyphagous attitude of the seed bug, which has been observed feeding on *P. abies* and, for the first time in the forest, on *P. nigra*, *P. sylvestris*, *P. mugo* and *L. decidua*. This ability seems particularly interesting, as pine stand distribution in Trentino is patchy and interspersed with other conifer species. It is therefore likely that the seed bug moves between islands of host conifers, as suggested by Gall (1992), leaning on the different available host species. Actually, overwintering adults were found in an alpine environment (Val Rendena) in a wood pile near a *P. abies* and *L. decidua* stands, where pines are absent. Therefore, all the observations conducted so far show an actual ability of *L. occidentalis* in colonizing high-altitude sites where the conifer species typical of subalpine belt are present.

This ecological adaptability is joined to a considerable plasticity in life cycle. Observations conducted both in the field (areas A_1, A_2 and A_3) and under artificial conditions showed that in southern Trentino *L. occidentalis* may produce one or two generations per year, depending on elevation and climatic conditions. A partial third generation was noticed in bottom valley areas in 2009. This suggests that in the future *L. occidentalis*, favoured by climate change, has the potential to complete three generations in southern Trentino. Although few researches have been conducted on the biology and the ecology of *L. occidentalis*, a similar voltinism was reported in northern Italy. In Appiano Gentile (Lombardia, nortwestern Italy) and Lignano (Friuli-Venezia Giulia, northeastern Italy), the seed bug completes two and three generations, respectively (Bernardinelli *et al*., 2006). These observations confirmed the same plasticity observed in the American region, with the difference that in northern California and southern British Columbia, similar to northern Italy in the latitude, *L. occidentalis* completes only one generation per year (Bates *et al*., 2002b; Koerber, 1963). These apparent contradictions could be explained either by a different adaptation capability of the seed bug to the new environment or by microclimatic conditions.

Heat accumulation appears to be the limiting factor for the development of *L. occidentalis*. The DD method proposed by Bernardinelli *et al.* (2006), whose validity was assessed in Trentino region conditions, seems a useful tool to predict the voltinism and the development rate of this insect. Even if only few data are available at present, we can conclude that the observed Δ values are in agreement with the predicted ones, especially under artificial conditions. Nevertheless, the not complete consistency between predicted and field-observed Δ values indicates that some parameters still have to be adjusted.

Adults' behaviour seems influenced by temperature thresholds, which trigger the coming in and out of overwintering and the trophic activity, as already suggested by Agostini *et al.* (2004). The correlation between mean temperatures and the dates in which the seed bug comes in and out overwintering seems to strengthen the hypothesis of threshold temperatures.

L. occidentalis has become familiar to common people especially for its overwintering habits. Besides natural shelters, adults can be found in buildings, unheated sheds and woodpiles. Only adult stages were found overwintering inside man-made structures, as Strong (2006) described too. The variable amounts of individuals found in artificial shelters overlooking forests lets suppose that this is not a need but an opportunity for the seed bugs. This habit, especially during fall and with high population densities, makes this species well-known to people, who feel it as a pest. Actually, a direct damage to human beings has not been demonstrated and *L. occidentalis* represents rather a nuisance (Blatt, 1994; Gall, 1992).

The studies performed so far in Europe have not yet reported either critical levels for the seed bug populations or economic harms, with the possible exception of Italian stone pine seed production (Benassai *et al*., 2007; Beránek, 2007; Hellrigl, 2006; Steyrer and Perny, 2007). More relevant could be the effect of this invasive species on alpine forest ecosystems, where this bug appears to be well established.

The potential involvement of *L. occidentalis* in vectoring *D. pinea* conidia was verified in this research by the microscope observation of relatively high percentages (from 35.0 to 42.5%) of positive samples, confirming the survey carried out in Tuscany (Italy) by Luchi *et al.* (2012). The fact that insects were collected during overwintering, and not directly from pine crowns, strengthens the hypothesis that at least a certain number of conidia, trapped in the hairiness of the seed bug during feeding on conelets, could be retained during overwintering. As a consequence, *L. occidentalis* could be able to spread the pathogen to new host plants in the following season. As *D. pinea* produces fruit bodies on two-year cones or on dead shoots, which are not the favourite *pabulum* of the insect, the contact can be accidental or due to the spore release. Moreover, *L. occidentalis* can infect directly the new cones and shoots, thus enhancing the pathogen spread in healthy parts of the plants.

D. pinea is an endophytic fungus in green tissues and symptom appearance is conditioned by stress (Maresi *et* *al.*, 2007). Bug attacks on conelets and needles could therefore be stress factors giving rise to a new colonization. The behaviour of the seed bug in carrying *D. pinea* conidia is similar to that of *Gastrodes grossipes* (De Geer), a lygeid feeding on pine cones (Feci *et al.*, 2002).

Finally, the western seed bug appears to be a good example of an invasive species adapting quickly to a heterogeneous mountain habitat. Its impact, however, is difficult to assess because of the constraints in population growth that could be associated with a mismatching between the developmental time and the local climatic conditions. There are, however, other factors that can be explored and may explain the variability in the colonization success. Among these, the association with other organisms such as the fungus *D. pinea* may play an important role, but their relationships with the colonization and population dynamics of the seed bug need to be further elucidated.

Acknowledgements

We thank the Università di Padova - Progetti di Ateneo for partial support to this work and Amelia Caffarra (FEM-IASMA) for the technical support.

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Received December 7, 2011. Accepted May 24, 2012.

Appendix

Field observations partially confirmed the results obtained under artificial conditions, demonstrating the polyphagy of *L. occidentalis* also in Trentino region. The seed bug was observed in the forest feeding on *P. nigra* (**A**, **B**), *P. sylvestris* (**C**), *P. mugo* and *L. decidua* (**D**), while feeding on *P. abies* was noticed only in artificial conditions (**E**). Nevertheless, not all the plant species offered during rearing resulted suitable for reproduction. A mating behaviour followed by oviposition was recorded only on *P. abies* and *P. mugo*. Egg masses were observed on *P. mugo* also in natural conditions, as shown in (**F**). Nymphal instars survived a certain period on *P. abies* and *P. mugo*, but they completed the development only on the latter species. The observations on the larch are not reliable because its needles are prone to desiccation. As already shown for *Chamecyparis lawsoniana* (Murray) Parl., another *Cupressaceae* species, *C. sempervirens* seems not suitable for the development of the seed bug (Koerber, 1963).
(**G**) Newly emerged adult, characterized by more bright and gaudy colours compared to overwintered ones. (**H**) Nymphs crouching in fall (10/28/2008) on *P. nigra* in area A_2. Between the end of October and the beginning of November, when a drop in population was noticed, only few newly developed adults and nymphs were lying close to conelets, without feeding. After few days adults had left the trees, while nymphs were still crouching. (**I**) Adult feeding on opened cone in artificial condition, as described also by Cibriàn-Tovar *et al.* (1986). (**J**) Conidium of *S. sapinea* detected on a positive sample.


A


B


C


D

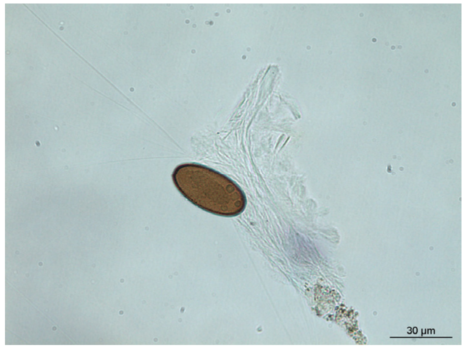

E


F


G


H


I



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