

Spatial clustering of *Diabrotica virgifera virgifera* and *Agriotes ustulatus* in small-scale maize fields without topographic relief drift

Stefan Toepfer¹, Michael M. Ellsbury², René Eschen¹ & Ulrich Kuhlmann^{1*}

¹CABI Europe – Switzerland, Rue des Grillons 1, CH-2800 Delémont, Switzerland, ²USDA-ARS, North Central Agricultural Research Laboratory, 2923 Medary Avenue, Brookings, SD 57006, USA

Accepted: 31 January 2007

Key words: western corn rootworm, Coleoptera, Chrysomelidae, western click beetle, Elateridae, soil, vegetation, variograms

Abstract

The soil-living larvae of *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) and *Agriotes ustulatus* Schaller (Coleoptera: Elateridae) can cause economic damage to maize roots, *Zea mays* L. (Poaceae). This study investigated the spatial clustering of both pests in four small-scale maize fields in southern Hungary, where clustering had been observed but not expected due to the lack of topographic relief drifts and soil structuring. Between 2000 and 2002, numbers of *D. v. virgifera* larvae and adults and of *A. ustulatus* larvae were determined at four randomly chosen georeferenced maize plants in each of 24 plots per field. Soil moisture, soil bulk density, and vegetational characteristics were assessed. Moran's I test for spatial autocorrelations, semivariogram analyses, and interpolated mapping revealed that *D. v. virgifera* larvae and adults were spatially clustered in 67 and 50% of cases, respectively. Larvae of *A. ustulatus* were clustered in 75% of cases. *Diabrotica virgifera virgifera* larval distributions were mainly determined by increasing weed density (negative correlation), in particular with high densities of *Cirsium arvense* (L.) (Asteraceae), as well as by increasing soil moisture (negative correlation). Adult distributions of *D. v. virgifera* were mainly determined by the density distribution of flowering maize. They were moreover correlated with larval distribution and with the adult distribution of the previous year. The density distributions of male adults differed from those of females. Female density was additionally correlated with higher soil moisture and Poaceae density, e.g., with *Sorghum halepense* (L.) Pers. No relation was found between the larvae of *A. ustulatus* and *D. v. virgifera*. *Agriotes ustulatus* larval distributions were mainly determined by vegetational cover (correlation with less cover). Conclusively, male and female *D. v. virgifera* adults, larvae of *D. v. virgifera*, and larvae of *A. ustulatus* will display different spatial clustering even within ostensibly homogeneous habitats of flat small-scale maize fields.

Introduction

The larvae of the western corn rootworm [*Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae)] as well as of the western click beetle [*Agriotes ustulatus* Schaller (Coleoptera: Elateridae)] are serious root feeding pests of maize, *Zea mays* L. (Poaceae). *Diabrotica virgifera virgifera* is invasive to Europe, having been accidentally introduced several times from North America between the

late 1980s and early 2000s (Miller et al., 2005). It currently reaches high population levels throughout central – south-eastern Europe and northern Italy (Kiss et al., 2005b). *Diabrotica virgifera virgifera* has one generation per year and overwinters as eggs in the soil (Krysan & Miller, 1986). In central Europe, the larvae emerge from eggs in May and the three instars feed nearly exclusively on maize roots (Moeser & Vidal, 2004a). Larvae may cause such extensive damage to maize roots that plants become susceptible to lodging. Reduced yields result from poor harvestability and physiological loss from destruction of roots (Chiang, 1973). The adults emerge between the end of June and early-August (Toepfer & Kuhlmann, 2006) and occasionally

*Correspondence: Ulrich Kuhlmann, CABI Europe – Switzerland, Rue des Grillons 1, CH-2800 Delémont, Switzerland.
E-mail: u.kuhlmann@cabi.org

reduce yields by interfering with pollination when feeding on silks.

Agriotes ustulatus is native to Europe. One generation lasts for about 24 months and has 12–13 instars (Furlan, 1998). Its larvae overwinter in the soil and can cause economic damage to crops after they reach the seventh instar in the summer of their second year (Furlan, 1998). Although larvae are polyphagous they can feed on maize roots in the same manner as do larvae of *D. v. virgifera* (Furlan, 1996) and can cause comparable economic damage. Adults emerge between June and September and live for 1–2 weeks, feeding on flowers of weeds, such as Apiaceae, but do not damage crops (Furlan, 1996).

As with many pest populations, *D. v. virgifera* and *A. ustulatus* were shown to be often spatially clustered (Ellsberry et al., 1998; Furlan & Burgio, 1999; Parker & Howard, 2001). This fact reduces the precision of sampling methods for population measures (Iwao, 1970; Furlan & Burgio, 1999) and complicates the establishment of reliable thresholds for control measures (Ellsberry et al., 1998). Geostatistical analyses of spatial patterns and clustering can help to adjust sampling methods, and to understand key factors that determine population size and dynamics of such pests (Liebhold et al., 1993).

Reasons for the clustering of insect distributions can be found (i) in environmentally mediated mortality factors that act on pest populations, which may change spatially as well as seasonally (Ellsberry et al., 1998); and (ii) in biological factors such as sex pheromones (Raemisch & Turpin, 1984; Schalk et al., 1990) or limited movement of larvae (Ellsberry et al., 1999b). In contrast to *A. ustulatus*, many studies at different population levels or at different scales have already reported such spatial dependencies for *D. v. virgifera* or other Diabroticite pests, especially from North America (Steffey & Tollefson, 1982; Bergman et al., 1983; Ellsberry et al., 1996, 2004; Darnell et al., 1999).

On a regional scale, Beckler et al. (2004) reported that *D. v. virgifera* populations are always clustered and their densities depend mainly on the size and arrangement of maize fields and on regional differences in soil texture or elevation. Also *Agriotes* spp., like *Agriotes lineatus* L. or *Agriotes obscurus* L., were reported to be clustered into patches and gaps of landscapes (Blackshaw & Vernon, 2006).

At the within-field scale of large fields of 10–1000 or more hectares, eggs of *D. v. virgifera* may be randomly distributed (Ellsberry et al., 1997; Park & Tollefson, 2006a). In contrast, the distribution of larvae (Ellsberry et al., 1999a), of root injury (Park & Tollefson, 2005a), and of adults (Midgarden et al., 1993; Park & Tollefson, 2005b) was often found to be spatially clustered. Variation in spatial distribution was correlated with various factors, including

adult distribution during population peaks in previous years, or topographic features such as slopes, landscape position, or soil texture. However, the clustering may also disappear in *Diabrotica* populations at large densities (Steffey & Tollefson, 1982). Adults of the *Agriotes* pests, *Agriotes sordidus* Illiger and *Agriotes litigiosus* Rossi, were reported to be spatially clustered, but no explanation of factors behind this distribution was given (Burgio et al., 2005).

At smaller within-field scales or in small plots of 1–20 m, eggs (Foster, 1977; Park & Tollefson, 2006a), larvae, and adults of *Diabrotica* spp. (Park & Tollefson, 2005a,b) were often found to be clustered with respect to soil moisture, soil texture, pollen production, and silking (Darnell et al., 1999, 2000). The high mortality recorded in newly hatched first instars when searching for maize roots (Strnad & Bergman, 1987b) suggested that maize density also has an influence on larvae at this scale. Also, high densities of weeds and the carbon dioxide (CO₂) emission of their root systems may disrupt the orientation of first instars towards the emission of maize roots (Bjostad & Hibbard, 1992). Larvae of the *Agriotes* pests, *A. ustulatus*, *Agriotes brevis* Candeze, and *A. sordidus*, were also reported to be spatially clustered, but no explanation of factors behind their distribution was given (Furlan & Burgio, 1999).

At the plant level, Bergman et al. (1983) showed that *D. v. virgifera* larvae were associated with maize root systems, and Strnad & Bergman (1987a), Gustin & Schumacher (1989), and Ellsberry et al. (1994) demonstrated major influences of soil porosity and moisture on first instars. Darnell et al. (1999), Park & Tollefson (2005b), Hill & Mayo (1974), and Prystupa et al. (1988) revealed an increased concentration of adults on flowering plants and especially around silking cobs.

In central and south-eastern Europe, farmers and researchers are mainly concerned about distributions of pests within small-holder maize fields. Because many fields in this region are flat, with a homogeneous soil structure and a high weed diversity, observations of pest clustering have been difficult to explain (Kiss et al., 2005a). Moreover, the effect of various vegetation parameters on spatial patterns of the two key pests *D. v. virgifera* and *A. ustulatus* have never been investigated, although alternative host plants of *D. v. virgifera* larvae or alternative pollen sources for its adults have been intensively studied (Branson & Ortman, 1967, 1970; Moeser & Vidal, 2005), and a large range of host plants is known for *A. ustulatus* larvae and adults (Furlan, 1996, 1998). In order to understand key factors that determine spatial clustering of both pests, this study characterized their spatial distribution patterns in four small-scale maize fields with diverse, weedy vegetation and with neither topographic relief nor apparent variability in soil type or texture, as is typical for small-holder farming

in southern Hungary. Spatial analyses were conducted in a step-by-step manner, as suggested by Legendre & Fortin (1989), Krajewski & Gibbs (2001), Liebhold et al. (1993), and Rossi et al. (1993):

1. The presence of a spatial structure such as clustering of the data was analysed by the Z-test of Moran's I (Legendre & Fortin, 1989; Tiefelsdorf, 2002), which is an indicator of spatial autocorrelation.
2. Variogram analyses were applied to describe spatial structures, i.e., spatial autocorrelations (Legendre & Fortin, 1989).
3. Variogram results, i.e., the best-fitting variogram model, were used for drawing contour maps to visualize spatial patterns (Krajewski & Gibbs, 2001).
4. Plot-to-plot correlation analyses (Park & Tollefson, 2005b) were applied to determine the factors behind spatial pest distributions, such as relationships among pests' developmental stages or between the two pests *D. v. virgifera* and *A. ustulatus*, as well as relationships between pests and vegetational structure.

Materials and methods

Study areas

The study was carried out in Csongrad County, southern Hungary, from 2000 to 2002 in fields that had been continuously planted with maize (Hybrid Borbala, Hungary) for at least 4 years. All fields were naturally infested with *D. v. virgifera* and *A. ustulatus* and no insecticides had been applied. Field A (1.5 ha) was situated between Csanádpalota and Nagylak (46°11'938"N, 20°43'117"E). Fields B and C (1.5 ha) were adjacent and situated south of Csanádpalota (46°12'946"N, 20°44'267"E), 2.5 km north of field A, and 44 km east of field D (3 ha). Field D was situated south of Szeged (46°14'054"N, 20°08'936"N). Fields A, B, and C had a Csernozom soil of dense clay loam texture (typical grassland soil), and field D was a mollic fluvisol (typical alluvial soil) of dense clay loam texture.

A rectangular, 0.35 ha and flat study area was established centrally in each of the four study fields to avoid edge effects. Each area was systematically divided into 24 plots of 12 × 12 m. In each plot, four random samples were taken on each sample date (see next section), thus totalling 96 stratified random samples per date (Krysan & Miller, 1986; Southwood, 2000). For each sample, an x, y spatial coordinate was assigned using a tape measure with respect to a georeferenced (southwestern) corner (coordinates x, y = 0 m, 0 m) of the study area.

Diabrotica virgifera virgifera and *Agriotes ustulatus*

A soil-root sampling programme (Krysan & Miller, 1986; Southwood, 2000) to assess the density of *A. ustulatus*

and *D. v. virgifera* larvae was carried out every 10–14 days during June and July, i.e., 3–4 dates in each of two subsequent years in each field. On each sample date and in each of the 12 × 12 m plots, a golf course cup cutter (100 mm in depth × 120 mm in diameter, volume 1.1 l) was used to obtain four random soil cores containing a maize root (Bergman et al., 1981). This totalled 96 soil-root samples taken on each sample date. Each sample was crumbled by hand over black plastic sheets; roots were then dissected using a scalpel and visually searched for larvae of *D. v. virgifera* and *A. ustulatus*. Roots and soil were transferred to 5 mm mesh screens, which were then placed over moist paper. The remaining larvae fell on to the moist paper below the screen as roots dried out over 3 days. Numbers of larvae per maize plant were counted, and the position of each plant was spatially referenced to an x, y coordinate. Mean larval density per plant was calculated for each plot.

Adult densities of *D. v. virgifera* were recorded during a counting programme on whole maize plants. Recording commenced during first adult emergence at the end of June and continued on a weekly basis until the end of August. Thus, there were a total of 6–8 sample dates per year. In each plot, four randomly chosen maize plants were visually searched for adult *D. v. virgifera* from the bottom to the top, inside the ears, silks, and leaf base. Adults were collected using aspirators and sexed with the aid of a stereomicroscope (Chiang, 1973). A total of 96 counts were made on each sample date and an overall number of 576–864 counts were made per field and year. Average adult density (both sexes, males, and females) per plant per plot was calculated over the whole season, as well as early in the season (end of June, early July), during the population peak (second–sixth week after start of emergence, July), and late in the season (sixth–tenth week after start of emergence, end of July until end of August).

Soil and vegetation

To determine the spatial distribution of soil moisture and soil bulk density, a soil sampling programme was carried out in field B on 22 July and 21 August 2000; in field C on 29 July and 19 August 2002; and in field A on 24 July and 1 August 2002.

Four undisturbed soil cores were taken randomly in each of the 24 plots per field on each sample date (Copper cylinders, 50 mm in depth × 50 mm in diameter, volume 0.1 l). Each soil core was spatially referenced to an x, y coordinate. Soil cores were taken at a 7–12-cm depth. Cylinders with soil were immediately sealed with plastic lids and were transported to the laboratory. Samples were weighed, dried at 80–120 °C for 24 h, and then reweighed. Gravimetric soil moisture (h_v) and soil bulk density (ρ_s)

	Field A in 2002		Field C in 2002	
	Mean	SD	Mean	SD
Number of plants per m ²				
<i>Zea mays</i> L.	2.67	0.71	4.56	0.60
<i>Amaranthus retroflexus</i> L.	5.34	4.21	0.01	0.03
<i>Setaria glauca</i> (L.) Beauv. ¹	1.94	1.78	0.02	0.05
<i>Sorghum halepense</i> (L.) Pers.	1.80	2.90	0.04	0.15
<i>Convolvulus arvensis</i> L.	1.56	1.43	0.17	0.40
<i>Chenopodium album</i> L.	1.12	0.75	0.01	0.02
<i>Datura stramonium</i> L.	0.96	1.18	1.01	0.90
<i>Cirsium arvense</i> L.	0.61	1.10	1.89	2.06
<i>Xanthium orientale</i> L. ¹	0.33	0.53	0.01	0.01
<i>Chenopodium hybridum</i> L.	0.16	0.21	0.01	0.02
<i>Sonchus arvensis</i> L. ¹	0.10	0.38	0.001	0.00
Vegetational cover (%)	67.7	17.45	19.9	6.10
Weed species numbers per plot	14.6	1.44	7.1	1.42
Weeds per plot per m ²	14.7	4.29	4.8	2.50
Poaceae per plot per m ²	6.4	3.06	6.2	1.68

¹Not flowering at sample dates.

were calculated as h_s (weight %) = $a - b/b - c \times 100$ and ϕ_s ($g\ cm^{-3}$) = $m_2 - m_1/V$ (Barczi et al., 1991), where a = wet weight of the soil with ring and lids; $m_2 = b$ = dry weight of the soil with ring and lids; $m_1 = c$ = dry weight of the soil; V = volume of the soil core. Impact of rainfall or dry periods on the absolute data was minimized by using proportions of the data to calculate the means for the two sample dates, which were then used for the statistical analyses (Krebs, 1999).

To determine the vegetation characteristics, all weed species were identified. The numbers of weed specimens were counted as well as the number of maize plants within and between five maize rows in each of the 24 plots of the fields C on 26 July and A on 8 August 2002 (Table 1). Maize was sown at a distance of 0.2 m from one plant to the next in rows 0.75 m apart (around 67 000 plants per hectare). Not all plants grew, and thus the maize density differed within and between fields (Table 1). The percentage aerial cover by each weed species and maize was visually estimated. The mean density of weeds and maize of the five rows was calculated as a mean per square meter for each plot. The flowering status was determined for each maize plant on which *D. v. virgifera* adults had been counted over the whole sampling season (see above).

Analyses of spatial characteristics

Spatial analyses were conducted with the variable data (z) and corresponding spatial data (x, y) for the following: (i) the average density of *D. v. virgifera* larvae from May to June, (ii) the average density of *D. v. virgifera* adults early

Table 1 Vegetation parameters of two maize fields (A and C) in southern Hungary in 2002 (most abundant weed species out of 27 presented, data in field C from 26 July and in field A from 8 August 2002, number of weed species presented per 12 × 12 m plot)

in the season, during their population peak, and late in the season, (iii) the larval density of *A. ustulatus* from May to June, (iv) soil moisture and soil bulk density in July and August, and (v) for vegetational cover, weed species numbers, total weed densities, maize density, distribution of flowering maize, and the density of each weed species in July and August.

The presence or absence of spatial structure was tested by the Z-test of Moran's I using Crime Stat Software (Levine, 2004). Moran's I is a classic indicator of spatial autocorrelation, being an index of covariation between different point locations, and is similar to a product moment correlation coefficient, varying from -1 to $+1$. Its significance was tested under the assumption of randomization (Z-test), which indicates whether the differences between I values of sample data and the expected I are greater than would be expected by chance (Tiefelsdorf, 2002; Levine, 2004).

Variogram analyses were then applied to describe spatial structures of the autocorrelated data (Legendre & Fortin, 1989; Bjoernstad & Falk, 2001) using GS+ (Gamma Design Software, 2004). Prior to the analyses, sample data were transformed by the square root function to distribute data normally (after histogram analyses). Variograms plot distances between sample pairs against the semivariance statistic. The semivariance (γ) for n sample pairs was calculated as $\gamma(h) = (1/2n(h)) * \sum (z_i - z_{i+h})^2$, where h is the lag distance between samples for variable z . Active lag distances of 45–70 m were chosen, which is about one-third of the diagonal extent of the data (Surfer, 2002). Lag

class distance intervals of 4.5–6 were applied, depending on the study field, resulting in about 10–13 distance intervals. The value of the semivariance at the first (smallest) lag distance, termed the experimental nugget C_0 , was used as a conservative estimate of the proportion of variability due to spatial structure (Williams et al., 1992). The range A_0 is the lag distance beyond which samples are considered spatially independent, and a short range showed that differences occurred over short distances and a long range differences over long distances (Krajewski & Gibbs, 2001). The corresponding value of the semivariance at this point was termed the sill ($C_0 + C$) and represented the combination of a nugget (C_0) and variance (C) attributable to spatial dependence estimated as percentage of variability = $C / (C_0 + C)$ (Ellsberry et al., 1998). A low sill indicated little differences between sample values (Krajewski & Gibbs, 2001).

The best autocorrelation model was applied to the isotropic ($= 0-360^\circ$) variogram by interpreting the model outputs in the following priority: (i) visual identification of model by interpreting sill, range, and nugget values; (ii) smallest residual sum of squares for the fit of model (RSS) (Isaaks & Srivastava, 1989); (iii) the highest correlation coefficient r^2 ; (iv) highest proportion of $C / (C_0 + C)$, i.e., the proportion of sample variance or sill ($C_0 + C$) that is explained by the spatial structure variance C (Rossi et al., 1993; Ettema & Wardle, 2002; Gamma Design Software, 2004). Those interpretations were visually verified by drawing contour maps of interpolated sample data. Data were gridded by interpolations with kriging (Krajewski & Gibbs, 2001; Surfer, 2002). The kriging was based on the best-fit variogram models (see above).

Correlation among factors

Spatially structured factors (see Moran's I and variogram models in Tables 1 and 2) were analysed on a plot-to-plot basis for statistical correlation within years, between subsequent years and among factors (Kinnear & Gray, 2000; Park & Tollefson, 2005b). Sample data had been averaged per plot, and standardized by converting values to proportions. Then, data were explored for outliers using box plots, and extreme values of more than three box lengths above the box were identified and deleted. A matrix of scatter plots was drawn to see where linear relationships among factors were apparent (plots not shown). Where linear relationships were apparent, regression analyses were applied and the strength of linear associations was measured by the Pearson's correlation ($-1 < r < +1$). High, positive correlations between factors, that is, an r closer to 1, suggested that the spatial density distributions were more likely to be similar (Park & Tollefson, 2005b).

Results

Diabrotica virgifera virgifera and *Agriotes ustulatus*

Distributions of *D. v. virgifera* larvae were spatially influenced and clustered in four out of six maize fields or years (67%) (see Moran's I and variogram models Table 2). Larval densities showed spatial dependence over relatively long distances (ranges A_0 of 22–68 m, Table 2). An average (\pm SD) of 0.8 ± 0.7 and 0.1 ± 0.3 larvae per plant were found in field A in June 2001 and 2002, respectively. In field B, 0.2 ± 0.4 larvae per plant were found in June 2000; in field C 0.15 ± 0.18 and 0.2 ± 0.5 larvae per plant were found in 2001 and 2002, respectively; and 0.1 ± 0.1 larvae per plant were found in field D in 2000. In each field some areas were uninfested and other areas were heavily infested (Figure 1). A mean (\pm SD) of 2.8 ± 1.2 and 1.5 ± 0.9 larvae per plant were found in the more heavily infested areas of field A in 2001 and 2002, respectively. Heavily infested areas in field B had 1.8 ± 0.8 larvae per plant in 2000; in field C 0.5 ± 0.4 larvae per plant in 2001 and 2.5 ± 1.4 in 2002; and in field D 0.5 larvae per plant in 2000. The differences between these densities were relatively low, reflected by the low sill values $C_0 + C$ of the semivariograms (Table 2).

Density distributions of *D. v. virgifera* adults were less often spatially influenced than those of larvae. Adult distributions were clustered in three of six maize fields (see Moran's I and variogram models Table 2). Adult densities showed strongest spatial dependencies over longer distances (ranges A_0 of 25–78 m, Table 2). Comparable results were obtained when females and males were analysed separately (Table 2). In field A during July 2001 and 2002, 0.6 ± 0.5 adults per plant (max = 2.2 in 2001, max = 2.5 in 2002) were found. In field B, 0.3 ± 0.4 adults per plant (max 2.5) were found during July 2000. In field C, 0.07 ± 0.1 (max = 0.5) and 0.2 ± 0.3 adults per plant (max = 1.3) were found in 2001 and 2002, respectively, and in field D, 0.6 ± 0.5 adults per plant (max = 2.2) were found in 2000. Across the sites A and B and years 2001 and 2000 of sampling, respectively, an average of 47% females and 53% males (± 1.2) were found.

Density distributions of *A. ustulatus* larvae were clustered in three out of four maize fields or years (see Moran's I and variogram models Table 2). Larval densities showed strongest spatial dependencies over short distances (ranges A_0 of 7–9 m, Table 2). Numbers of larvae per plant differed greatly within a field (see high or low sill values, Table 2). In field A in 2001 and 2002, 0.7 ± 0.9 (max = 3.9) and 0.1 ± 0.1 (max 0.5) larvae of *A. ustulatus* were found per maize plant, respectively. In field C, 0.1 ± 0.1 (max = 0.5) and 0.05 ± 0.1 (max = 0.3) larvae per plant were found in 2001 and 2002, respectively (Figure 1).

Table 2 Spatial characteristics of *Diabrotica virgifera virgifera* and *Agriotes ustulatus* according to spatial autocorrelation (Moran's I) and to the best-fit variogram model (Isotropic = 0–360° variogram using semivariances are presented)

Factor	Field	Year	Dates ¹	n ²	Moran's I ³	P (Z-test) ⁴	Model of variogram	RSS ⁵	Nugget ⁶	Sill ⁷	Range (m) ⁸	r ²	P
<i>Diabrotica v. virgifera</i> larvae	A	2001	4	96	0.49	**	Spherical	0.12	0.001	0.37	22	0.49	**
	A	2002	3	96	-0.03	ns	Linear	0.002	0.05	0.05	44	0.00	ns
	B	2000	4	96	0.14	**	Spherical	0.00008	0.11	0.15	68	0.99	**
	C	2001	4	96	0.35	**	Spherical	0.001	0.0001	0.006	29	0.79	**
	C	2002	3	96	0.03	ns	Linear	0.00005	0.038	0.04	33	0.14	ns
	D	2000	4	96	0.07	**	Exponential	0.00005	0.009	0.02	6.6	0.47	**
<i>Diabrotica v. virgifera</i> adult females	A	2001	5	96	0.12	**	Linear	0.0013	0.125	0.15	42	0.23	*
	A	2002	6	96	0.004	ns	–						
	C	2001	8	96	-0.09	ns	–						
	C	2002	6	96	-0.03	ns	–						
<i>Diabrotica v. virgifera</i> adult males	A	2001	5	96	0.19	**	Exponential	0.0017	0.052	0.13	13	0.69	**
	A	2002	6	96	-0.002	ns	–						
	C	2001	8	96	0.015	ns	–						
	C	2002	6	96	-0.002	ns	–						
<i>Diabrotica v. virgifera</i> adults both sexes	A	2001	5	96	0.19	*	Exponential	0.0013	0.06	0.16	25	0.77	**
	A	2002	6	96	0.02	ns	Spherical	0.012	0.06	0.23	3.2	0.09	ns
	B	2000	7	96	0.11	**	Spherical	0.00008	0.03	0.1	41	0.96	**
	C	2001	8	96	0.0003	ns	Linear	0.0005	0.038	0.041	33	0.14	ns
	C	2002	6	96	0.01	ns	Linear	0.0005	0.12	0.12	43	0.00	ns
	D	2000	8	96	0.11	**	Gaussian	0.00012	0.05	0.37	78	0.99	**
<i>Agriotes ustulatus</i> larvae	A	2001	4	96	0.53	**	Spherical	0.41	0.001	0.71	25	0.59	**
	A	2002	3	96	0.008	ns	Linear	0.00002	0.016	0.016	3.4	0.21	*
	C	2001	4	96	0.28	**	Spherical	0.00099	0.0001	0.04	9.3	0.6	**
	C	2002	3	96	0.16	**	Spherical	8.8×10^{-6}	0.0006	0.009	7.3	0.7	**

¹Number of sampling dates per year.

²Number of samples per date.

³Spatial autocorrelation coefficient indicates spatial clustering.

⁴Randomization significance (Z-test, two-tailed) of Moran's I: * <0.05 , ** <0.005 , ns, not significant.

⁵Residual sum of square for the fit: Model with lowest RSS was chosen as best-fitting variogram model.

⁶Nugget variance (C_0).

⁷Sill = $C_0 + C$: e.g., a low sill reflects little difference between sample values.

⁸Range = A_0 : a short range reflects that value differences occur over short distances.

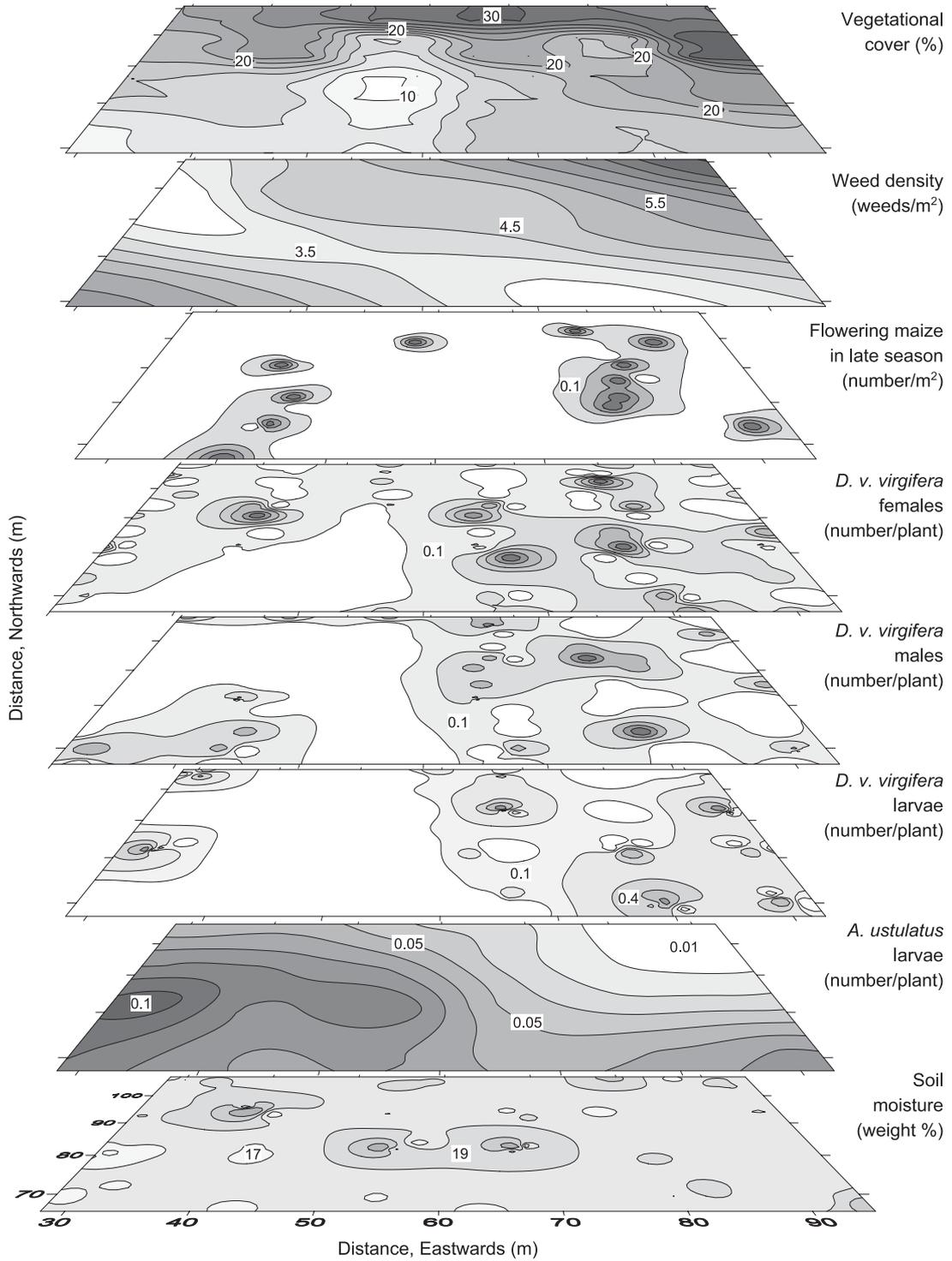


Figure 1 Contour maps showing interpolated density distributions of larvae and adults of *Diabrotica virgifera virgifera* and of larvae of *Agriotes ustulatus* mapped in comparison to soil moisture, vegetation cover, and weed density in maize field C in southern Hungary in 2002 (gridding of data using interpolations by kriging based on the best-fit variogram models from Tables 2 and 3).

Soil and vegetation

Soil bulk density distributions were not clustered (Table 3). In field A in 2002, bulk density ranged from 1.0 to 1.4 g cm⁻³ [mean (\pm SD) of 1.22 \pm 0.08], in field B in 2000 from 1.0 to 1.5 g cm⁻³ (1.27 \pm 0), and in field C in 2002 from 1.0 to 1.6 g cm⁻³ (1.34 \pm 0.1). Soil moisture was clustered in two of three fields (Table 3) in which spatial dependencies were found over shorter distances (ranges A_0 of 4–7 m; Table 3). The soil moisture differed between areas in the maize fields (see high sill values; Table 3). In field A in 2002, the soil moisture ranged from 11.1 to 19.0 weight percentage [mean (\pm SD) of 14.8 \pm 1.6], in field B in 2000 from 10.6 to 13.8 weight percentage (15.8 \pm 1.3), and in field C in 2002 from 13.8 to 41.4 weight percentage (18.5 \pm 3.6).

Vegetational cover was clustered in both maize fields investigated (Table 3). Strong spatial dependencies were found over short and relatively long distances (ranges A_0 of 15–64 m; Table 3). The mean vegetational cover differed between fields A and C, i.e., 67 vs. 20% (Table 3). Within both fields, the vegetational cover values also differed to a great extent (see high sill values, Table 3). Vegetational cover ranged from 45 to 100% in field A and from 10 to 31% in field C. Differences in vegetational cover were mainly a result of abundant broad-leaf weeds, e.g., *Amaranthus retroflexus* L. and *Chenopodium album* L., as well as of the abundant and tall *Sorghum halepense* (L.) (Table 1). A total of 27 weed species was found, 16 in field C (Table 1) and 21 in field A in 2002. The mean densities of the most abundant weed species are presented in Table 1. The number of weed species within a maize field also greatly differed (see high sill values, Table 3). In field A, the number of weed species ranged 12–17 per plot [mean (\pm SD) of 14.6 \pm 1.4] and in field C ranged 4–9 species (7.1 \pm 1.4). The weed density values differed between plots within a maize field (see high sill values; Table 3). Weed density ranged 5–25 per plot [mean (\pm SD) of 15 \pm (4)] in field A and 1–10 (5 \pm 2.5) in field C. Weed densities and number of weed species were clustered in both fields (Table 3). Spatial dependencies in the distribution of the number of weed species occurred over longer distances (see ranges A_0 of approximately 30 m; Table 3). Weed species were spatially clustered when analysed separately (only *Cirsium arvense* shown in Table 3).

Although, about 67 000 maize plants had been sown per hectare; not all plants grew, and thus the maize density differed within and between fields (Table 1). In field A, 2.8 maize plants per m² were counted and in field C, 4.8 plants per m². The maize density distribution (Table 3) and distribution of flowering maize plants were always spatially clustered on each sample date (data not shown), but rarely if averages over July and August were analysed (Table 3).

Correlations between factors

The density distributions of *D. v. virgifera* larvae were neither correlated with each other over subsequent years (Table 4), nor were they correlated with the distribution of adults in the previous year (Table 4). A higher density of larvae was correlated with lower soil moisture (Table 4 and Figure 1). *Diabrotica v. virgifera* larval densities were not correlated with vegetational cover or weed species numbers, however, they were negatively correlated with increasing weed density (Table 4). The latter was a result of the negative influence of high densities of *C. arvense*, whereas the density distributions of all other weed species as well as of maize were not correlated with the density of *D. v. virgifera* larvae.

Diabrotica v. virgifera adults were mainly found in plots of a maize field where higher larval densities had been found earlier in the summer of the same year (for both sexes combined, or females and males only, Table 4). Furthermore, the adult density distribution was positively correlated with the adult distribution over subsequent years (Table 4). The density distributions of adult *D. v. virgifera* remained consistent between sample dates from the end of June to the end of August of the same year, although absolute population levels changed ($r = 0.5\text{--}0.8$, $P > 0.05$; $n = 143$). When considering both sexes together, the density distribution of adult *D. v. virgifera* was neither correlated with soil moisture nor with vegetation cover, with number of weed species nor weed density, nor with the density of maize or any of the weed species found (Table 4). When sexes were analysed separately, male and female density distributions appeared to be different, i.e., they were not correlated with each other. The density of *D. v. virgifera* females was positively correlated with soil moisture, with highest frequencies of individuals occurring in areas with a high density of Poaceae, that is, mainly *S. halepense* (Table 4 and Figure 1). *Diabrotica v. virgifera* densities of both sexes, as well as females and males separately, were correlated with the density distribution of flowering maize plants. The effect was especially strong later in the season and stronger for females than for males (Table 5).

No correlation was found between *A. ustulatus* larval density distribution and *D. v. virgifera* larval density distribution (Table 4). *Agriotes ustulatus* larval density distribution was neither correlated with soil moisture, nor with weed density, maize density, nor any single weed species. *Agriotes ustulatus* larval density was negatively correlated with denser vegetational cover (Table 4).

Discussion

Spatial clustering is a well-known phenomenon for nearly every life form (Remmert, 1992) and has been reported

Table 3 Spatial characteristics of soil and vegetation according to spatial autocorrelation (Moran's I) and to the best-fitting variogram model (Isotropic variogram using semivariances are presented for autocorrelated data)

Factor	Field	Year	Dates ¹	n ²	Moran's I ³	P (Z-test) ⁴	Model of variogram	RSS ⁵	Nugget ⁶	Sill ⁷	Range (m) ⁸	r ²	P
Soil bulk density	A	2002	2	96	0.07	ns	Linear	0.0004	0.006	0.008	45	0.4	*
	B	2000	2	96	-0.01	ns	Spherical	0.000003	0.003	0.008	5.7	0.3	ns
	C	2002	2	96	-0.03	ns	Gaussian	6.3×10^{-6}	0.006	0.01	1.8	0.15	ns
Soil moisture	A	2002	2	96	-0.28	**	Spherical	0.00036	0.009	0.039	6.7	0.41	**
	B	2000	2	96	0.07	*	Spherical	0.53	0.69	1.77	3.6	0.02	ns
	C	2002	2	96	-0.03	ns	Linear	0.009	0.14	0.14	44	0.00	ns
Vegetational cover	A	2002	1	24	0.08	**	Spherical	0.09	0.009	0.83	15	0.57	**
	C	2002	1	24	0.22	**	Spherical	0.0027	0.001	0.77	64	0.99	**
Weed species numbers	A	2002	1	24	0.11	**	Exponential	0.122	0.57	3.23	29	0.93	**
	C	2002	1	24	0.12	**	Exponential	0.089	0.46	2.93	32	0.94	**
Weed density	A	2002	1	24	0.13	**	Gaussian	4.6	0.11	19.9	10	0.96	**
	C	2002	1	24	0.06	**	Spherical	0.0055	0.001	0.36	16	0.8	**
Maize density	A	2002	1	24	0.09	**	Spherical	0.00006	0.0001	0.042	16.5	0.88	**
	C	2002	1	24	0.17	**	Exponential	0.0011	0.001	0.45	20	0.98	**
Flowering maize (late season) ⁹	A	2001	5	96	-0.02	ns	-						
	A	2002	6	96	-0.01	ns	-						
	B	2000	7	96	0.01	**	Spherical	0.00015	0.022	0.081	9.4	0.18	*
	C	2001	8	96	-0.005	ns	-						
	C	2002	6	96	-0.005	ns	-						
	D	2000	8	96	-0.008	ns	-						
<i>Cirsium arvense</i>	A	2002	1	24	0.13	**	Spherical	0.004	0.001	0.35	23	0.89	**
	C	2002	1	24	0.1	**	Spherical	0.01	0.17	0.65	44	0.9	**
Other weed species	A	2002	1	24	all spatially clustered, results not presented								
	C	2002	1	24	all spatially clustered, results not presented								

¹Number of sampling dates per year.²Number of samples per date.³Spatial autocorrelation coefficient indicates spatial clustering.⁴Randomization significance (Z-test, two-tailed) of Moran's I: * <0.05 , ** <0.005 , ns, not significant.⁵Residual sum of square for the fit: Model with lowest RSS was chosen as best fitting variogram model.⁶Nugget variance (C_0).⁷Sill = $C_0 + C$: e.g., a low sill reflects little difference between sample values.⁸Range = A_0 : a short range reflects that value differences occur over short distances.⁹Late season means from 6–10 weeks after start of emergence of *D. v. virgifera*.

Table 4 Correlation matrix among factors possibly related to *Diabrotica virgifera virgifera* larval density, to *D. v. virgifera* adult density, or to *Agriotes ustulatus* larval density in maize fields in Hungary

		<i>D. v. virgifera</i> larvae			<i>D. v. virgifera</i> adults									<i>A. ustulatus</i> larvae		
					Females			Males			Both sexes					
		r	P	n	r	P	n	r	P	n	r	P	n	r	P	n
Pests	<i>D. v. virgifera</i> larvae				0.25	0.01	96	0.23	0.01	96	0.22	0.00	143	0.00	0.50	96
	<i>D. v. virgifera</i> larvae in previous year	-0.14	0.18	48	-0.14	0.17	48	-0.15	0.15	48	-0.08	0.30	48	0.01	0.46	48
	<i>D. v. virgifera</i> adults in previous year	0.20	0.08	48	0.19	0.10	48	0.18	0.11	48	0.32	0.01	48			
	<i>D. v. virgifera</i> males				0.11	0.15	96				0.48	0.00	96			
	<i>D. v. virgifera</i> females							0.11	0.15	96	0.78	0.00	96			
	<i>A. ustulatus</i> larvae	0.00	0.50	96												
	<i>A. ustulatus</i> larvae in previous year	0.09	0.27	47										0.14	0.17	47
Soil	Soil moisture	-0.29	0.01	68	0.32	0.02	48	-0.12	0.21	48	-0.05	0.36	68	0.10	0.25	48
	Soil bulk density	not analysed because no spatial pattern had been found														
Vegetation	Weed density	-0.24	0.05	48	0.10	0.25	48	-0.01	0.46	48	0.15	0.16	48	-0.16	0.14	48
	Weed species numbers	0.06	0.36	48	0.23	0.06	48	-0.23	0.06	48	0.08	0.29	48	-0.06	0.34	48
	Vegetational cover	0.14	0.17	48	0.03	0.43	48	0.00	0.50	48	-0.05	0.37	48	-0.37	0.00	48
	<i>Sorghum halepense</i>	0.11	0.24	42	0.34	0.01	42	0.19	0.12	42	0.12	0.22	42	0.13	0.20	42
	<i>Amaranthus retroflexus</i>	0.21	0.08	46	0.00	0.49	46	0.14	0.18	46	0.19	0.11	46	-0.08	0.29	46
	Maize density	0.13	0.18	48	0.07	0.32	48	0.15	0.15	48	0.10	0.26	48	-0.02	0.45	48
	Density of all Poaceae	0.01	0.46	48	0.31	0.02	48	0.06	0.34	48	0.06	0.34	48	0.00	0.49	48
	<i>Chenopodium album</i>	0.10	0.26	46	-0.09	0.29	46	-0.01	0.46	46	-0.08	0.31	46	-0.17	0.14	46
	<i>Chenopodium hybridum</i>	-0.05	0.38	42	-0.08	0.30	42	-0.04	0.41	42	-0.13	0.20	42	0.20	0.11	42
	<i>Cirsium arvense</i>	-0.37	0.01	47	-0.12	0.22	47	-0.19	0.10	47	-0.16	0.14	47	0.20	0.09	47
	<i>Convolvulus arvensis</i>	-0.04	0.38	47	-0.20	0.09	47	-0.06	0.34	47	-0.13	0.19	47	0.11	0.23	47
	<i>Datura stramonium</i>	-0.05	0.37	48	0.00	0.49	48	-0.07	0.32	48	0.17	0.13	48	-0.15	0.15	48
	<i>Setaria glauca</i>	-0.09	0.28	47	-0.15	0.16	47	0.11	0.23	47	-0.04	0.40	47	-0.14	0.18	47
	<i>Sonchus arvensis</i>	0.19	0.12	40	-0.04	0.40	40	0.22	0.08	40	0.11	0.26	40	-0.13	0.21	40
<i>Xanthium orientale</i>	-0.22	0.09	41	-0.10	0.28	41	-0.23	0.08	41	0.03	0.42	41	0.11	0.24	41	

Regression analyses with r = Pearson's correlation coefficient; significance (one-tailed) for at P<0.05 shown in bold; n, number of sample pairs.

Table 5 Correlation matrix among *Diabrotica virgifera virgifera* adult density and flowering percentage of maize plants of four maize fields in southern Hungary

Flowering maize plants	<i>D. v. virgifera</i> adults														
	Females					Males					Both sexes				
	r	P	n	r	P	r	P	n	r	P	r	P	n		
Early season (first 4 weeks after start of adult emergence, end-June to mid-July)	-0.02	0.39	236	-0.07	0.15	0.05	0.16	380							
Peak of <i>D. v. virgifera</i> (2–6 weeks after start of adult emergence, July)	0.10	0.04	306	0.10	0.04	0.11	0.01	452							
Late season (6–10 weeks after start of adult emergence, end of July to end of August)	0.20	0.00	251	0.07	0.14	0.15	0.00	397							
Mean of all data (June, July, and August)	0.13	0.00	535	0.15	0.00	0.12	0.00	825							

Regression analyses with r = Pearson's correlation coefficient; significance (one-tailed) of r at P<0.05 shown in bold; n, number of sample pairs.

many times for insect pest populations (Price, 1984; Dent, 2000). Many studies have also reported such spatial dependencies for *D. v. virgifera* or other diabroticite pests, especially in North America, at different population levels and at different scales (e.g., Steffey & Tollefson, 1982; Bergman et al., 1983; Midgarden et al., 1993; Ellsbury et al., 1996, 2004; Darnell et al., 1999; Park & Tollefson, 2005b). However, many of the within-field scale studies could not fully explain spatial structuring, or were overlaid by topographic relief drift and soil texture variations. For *A. ustulatus*, explanations for its spatial structuring were totally lacking.

This study is unique in that it describes clustering of the two maize pests, *D. v. virgifera* and *A. ustulatus*, in maize fields that had no topographic relief drift and that were expected to have little spatial structuring of the soil. Indeed, the investigated soil bulk density, one of the soil factors that influences *Diabrotica* larvae (Ellsbury et al., 1994), showed no spatial structuring in the study fields. Unfortunately, some structuring in the soil of the study fields was still measured, i.e., the clustering in soil moisture, and its influence on the pest populations had to be considered. Whereas *A. ustulatus* larvae were not obviously influenced by soil moisture, the distribution of *D. v. virgifera* was affected. First, the larval density distributions were negatively influenced by moisture, although moist soil is often considered to enhance larval survival (Macdonald & Ellis, 1990; Park & Tollefson, 2006a). However, when a combination of high soil moistures and very dense soils occur, negative effects on larval movement and survival can be found (Suttle et al., 1967; Spike & Tollefson, 1988; Macdonald & Ellis, 1990). The very moist conditions in Hungary in spring in combination with dense clay loam soils are suggested to be the reason for the dependencies revealed in this study. Second, female adults of *D. v. virgifera* were positively correlated with soil moisture. This is an indication of their preference for moist habitats for oviposition (Kirk et al., 1968; Chiang, 1973; Gustin, 1979), which is most likely why the distribution data of males could not be explained by such a relationship.

Despite the differences in soil moisture, vegetation displayed the most obvious spatial structuring within the chosen study fields, as seen for example in the high diversity of weeds. This is a common feature for small-holder farms in the study area. Vegetation structures were thus expected to influence the pest populations since it is known that:

1. *Diabrotica v. virgifera* larvae are nearly restricted to maize plants (Moeser & Vidal, 2005) and could be negatively influenced by extensive root systems of weeds.
2. Newly hatched *Diabrotica v. virgifera* larvae usually follow CO₂ gradients emitted by maize roots (Bjostad & Hibbard,

1992), but their orientation may be disrupted by the CO₂ emission of roots of abundant weeds.

3. *Diabrotica v. virgifera* adults can feed on any pollen source and are known to feed on flowering weeds in late summer and may therefore be concentrated in such areas (Moeser & Vidal, 2005). They are also attracted by late flowering maize plants (Elliott et al., 1990), which could effect spatial distribution;
4. *Agriotis ustulatus* larvae are polyphagous with a preference for the inhabited crop (Furlan, 1998).

Surprisingly, only a few relationships were found between vegetation structure and pest populations in this study. Most obvious was the influence of the distribution of flowering maize plants on the *D. v. virgifera* adults (see also Darnell et al., 2000; Nowatzki et al., 2002). This relationship was found to increase as the growing season progressed, such that adults, in particular females, were concentrated in the few remaining flowering sections of the maize fields at the end of the summer. Females are known to continuously produce eggs (Chiang, 1973) and depend on a permanent food supply, thus explaining their attraction to the remaining flowering maize plants. Nevertheless, the large quantity of several flowering weed species, as well as the varying vegetational cover in the study fields, seemed to have no measurable effect on the distribution of *D. v. virgifera* adults even though such relationships were found for *Diabrotica undecimpunctata* (Brust & House, 1990). The only exception was that *D. v. virgifera* females were found to be concentrated in dense spots of *S. halepense*, most likely in search for food.

Aside from the influence of soil moisture and maize flowering, *D. v. virgifera* adult distributions are logically also determined by its larval distribution (Table 4; Ellsbury et al., 1998). Therefore, the major factors determining distributions of this pest should be examined on its soil-dwelling stages. However, *D. v. virgifera* larval distributions were not influenced by most of the weeds in this study, demonstrating that the larvae seem to locate the maize roots around those of the weeds, or that weed root systems were still too small to interfere with the newly hatched larvae in May. Only in areas with a high density of weeds, and especially in areas with abundant *C. arvensis*, was a negative effect observed on *D. v. virgifera* larvae. An influence of maize distribution on *D. v. virgifera* distributions was not reflected in the data likely because its level of spatial structuring was not sufficient.

Few or no correlations were found between subsequent years in this study, although Park & Tollefson (2005b) proved such correlations at least for the adults at high population levels or during population peaks. However, those studies also showed that adult density distributions

were affected by the maize phenology, and this may change from year to year. In this study, no relationship between larval distribution in one year and adult distribution in the previous year was found, even if only the time period of adult population peaks or the late season populations were considered. Eggs have been reported to often be randomly distributed (Ellsbury et al., 1997) on a larger scale but clustered on a small scale (Park & Tollefson, 2006a). Additionally, the high concentration of adults in late flowering maize spots suggests that oviposition may also be clustered. Therefore, the above-mentioned missing correlation shows that a possible clustering effect during the oviposition period in late summer can be diluted by spatially and seasonally changing mortality factors during the overwintering of eggs, or during the hatching and movement of first instars (Toepfer & Kuhlmann, 2005).

Finally, spatiality-determining factors may consist of a combination of (i) soil texture, e.g., soil bulk density (Ellsbury et al., 1994), and porosity (Gustin & Schumacher, 1989); (ii) soil moisture (Ellsbury et al., 1994); and (iii) maize or weed density (this study). Natural enemies seem to have little influence (Toepfer & Kuhlmann, 2004), and competition is negligible at normal densities (Branson et al., 1980). However, the effects of other underlying factors on the distribution of *D. v. virgifera* remain unknown, such as for example the influence of endophytic fungi (Moeser & Vidal, 2004b), maize root volatiles (Rasmann & Turlings, 2004), or predation during the egg stage (Brust & House, 1990).

When comparing the spatial distributions of the two soil-dwelling maize pests, *D. v. virgifera* and *A. ustulatus*, their ecological requirements seem to be different even though they inhabit the same habitat. For example, the larval distributions of *A. ustulatus* largely differed from the distribution of *D. v. virgifera* and depended, in contrast to *D. v. virgifera*, not on soil moisture or weed density, but on vegetational cover. Generally, few factors were found to influence *A. ustulatus* distributions, however, some relationships may have been overlooked due to the limitation of this study to several months per year instead on the whole development period of *A. ustulatus*, over more than 20 months. The 12–13 instars of *A. ustulatus* larvae (Furlan, 1996) live in the soil for a much longer time period than *D. v. virgifera*, and thus environmental factors may have more of an effect on this species than on the three instars of *D. v. virgifera*.

In conclusion, *D. v. virgifera* larvae were spatially clustered in most fields or years, and this clustering was determined to some degree by lower soil moisture and higher weed density. This larval distribution largely determined the distribution of *D. v. virgifera* adults that were clustered in half of the fields and years investigated. Adults were

also influenced by soil moisture and flowering maize. No relationship was found between the clustering of larvae of *A. ustulatus* and *D. v. virgifera*. Both species seem to have different ecological requirements within the same habitat. Therefore, even in small-scale maize fields, pest monitoring must account for clustering by either increasing sample sizes and distances between samples, or using stratified random sampling instead of random sampling, or using adaptive cluster sampling (Midgarden et al., 1993; Krebs, 1999; Park & Tollefson, 2006a,b). Moreover, the clustering of *D. v. virgifera* or *A. ustulatus* can not be reliably predicted over subsequent years on a small field scale.

Acknowledgements

We acknowledge the technical support of Marianna Szucs, the team at the Plant Health Service of Csongrad Country in southern Hungary and their cooperating farmers. We further would like to thank Emma Hunt (CABI – Switzerland), Ivan Hiltpold (University of Neuchatel Switzerland), and Kurt Rosentrater (USDA-ARS) for helpful discussion. We would also like to thank Joachim Moeser (University of Goettingen, Germany) for his help in determining weeds in Hungary. Finally, we would like to thank Lorenzo Furlan (University of Padova, Italy) for determining the elaterid larvae. The Federal Office of Science, Switzerland financed this work within the framework of the EU project DIABROTICA (QLK5-CT-1999-011110).

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