Climatic, Edaphic Factors and Cropping History Help Predict Click Beetle (Coleoptera: Elateridae) (Agriotes spp.) Abundance

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ABSTRACT. It is assumed that the abundance of Agriotes wireworms (Coleoptera: Elateridae) is affected by agro-ecological factors such as climatic and edaphic factors and the crop/previous crop grown at the sites investigated. The aim of this study, conducted in three different geographic counties in Croatia from 2007 to 2009, was to determine the factors that influence the abundance of adult click beetle of the species Agriotes brevis Cand., Agriotes lineatus (L.), Agriotes obscurus (L.), Agriotes sputator (L.), and Agriotes ustulatus Schall. The mean annual air temperature, total rainfall, percentage of coarse and fine sand, coarse and fine silt and clay, the soil pH, and humus were investigated as potential factors that may influence abundance. Adult click beetle emergence was monitored using sex pheromone traps (YATLORf and VARb3). Exploratory data analysis was performed via regression tree models and regional differences in Agriotes species’ abundance were predicted based on the agro-ecological factors measured. It was found that the best overall predictor of A. brevis abundance was the previous crop grown. Conversely, the best predictor of A. lineatus abundance was the current crop being grown and the percentage of humus. The best predictor of A. obscurus abundance was soil pH in KCl. The best predictor of A. sputator abundance was rainfall. Finally, the best predictors of A. ustulatus abundance were soil pH in KCl and humus. These results may be useful in regional pest control programs or for predicting future outbreaks of these species.

Key Words: Abundance, Agro-ecological factor, Click beetle, Prediction, Regression tree

Materials and Methods

Sample Sites. During three growing seasons (2007–2009) five Agriotes species (A. brevis, A. lineatus, A. sputator, A. obscurus, and A. ustulatus) were trapped in three different counties of Croatia representing three distinct climatic and edaphic areas (county 1: Koprivnica-Križevci, county 2: Virovitica-Podravina, county 3: Vukovar-Sirmium; Fig. 1).

Agriotes spp. specimens were collected from 15 fields sown with either corn Zea mays (L.), wheat Triticum aestivum (L.), barley Hordeum vulgare (L.), oats Avena sativa (L.), alfalfa Medicago sativa (L.), soybeans Glycine max (L.), sugar beet Beta vulgaris (L.), or white clover Trifolium repens (L.) (depending on the year and location). For each

Wireworms, the larvae of click beetles (Coleoptera: Elateridae), are cosmopolitan soil pests that attack corn, potatoes and many other important food crops throughout the world (Parker and Howard 2001; Brunner et al. 2005). In Croatia, the most economically damaging species of the Agriotes genus are: Agriotes brevis Cand., A. lineatus (L.), A. obscurus (L.), A. sputator (L.), and A. ustulatus Schall. (Maceljski 2002). Species within the Agriotes genus show perennival development, where the larval stage may last from 2 to 5 years in time (Furlan 1996, 1998; Parker and Howard 2001; Sufyan et al. 2013; Traugott et al. 2015). Based on their long life cycles, click beetles are usually divided into two groups (Furlan 2005, Bažok 2006). Species of the first group, which include A. brevis, A. lineatus, A. obscurus, and A. sputator, overwinter as larvae or as adults. After several years of development, the larvae of this group pupate and during late summer or early autumn, the adults complete their development and remain underground to overwinter (Ester et al. 2001, Gomboc et al. 2001, Toth et al. 2001). Adults then emerge between April and September the following year, depending on the species and geographic location (as influenced by climate, soil, and other microhabitat variables: Roebuck et al. 1947, Ester et al. 2001, Toth et al. 2001, Brunner et al. 2005, Landl et al. 2005, Vernon et al. 2005, Kozina et al. 2013). Species of the second group, which include A. ustulatus, overwinter only as larvae. Pupation takes place in May and adults emerge between May and September in the same year (Honek and Furlan 1995, Furlan 1996, Toth et al. 2001, Kozina 2012).

The beetles migrate to areas near their emergence place (Sufyan et al. 2007), however this distance may be greater than previously thought (e.g., 80 m for A. obscurus; Schallhart et al. 2009), allowing them to colonise new areas.

The preferred habitat for adult click beetles is usually in soils of grasslands, pastures, meadows and cultivated fields of alfalfa, white clover, sugar beet, or soybean (Furlan 1996; Čamprag 1997). Čamprag (1997) found a relationship between climatic factors and adult abundance, in which it was shown that adults form was greater when higher temperatures and lower rainfall prevailed. Due to their life cycle and the way in which they cause damage to crops, wireworms are pests whose suppression must be based on population level forecasts and on the principles of integrated pest management (IPM) (e.g., EU Directive 2009/128/EC). Determining the factors that positively or negatively affect the population growth of specific species under field conditions in particular counties of Croatia will facilitate the ability to forecast and manage outbreaks.

Therefore, the objectives of this study were: 1) to assess the abundance of five Agriotes species, which differ according to climatic and edaphic factors; and 2) for each species to determine the environmental variables by which its adult distribution and abundance can be predicted with the highest probability. To achieve these objectives, a robust predictive modelling technique using regression trees, was employed.
field, the crops sown the previous (hereafter referred to as precrop) and current years were recorded. The fields sampled were chosen so as to represent common cultivation and crop rotation practices in operation in each area. In western Croatia (county 1: Koprivnica-Križevci), arable crops (corn and soybean) and cereals (barley and wheat) are most commonly cultivated. In eastern Croatia, (county 2: Virovitica-Podravina region; and county 3: Vukovar-Sirmium region), a wider range of arable crops (corn, sugar beet, and soybean) and cereals (barley and wheat) are cultivated. Further details about the sampling sites are available in Supp Tables 1–3 (online only).

Climatic and Edaphic Factors. The three counties investigated were classified as belonging to the Cfwb x climatic type of the Köppen classification system (Penzar and Penzar 2000), where temperate (mesothermal) climates (Cf) with dry winters (w) dominate. The letter b indicates warmest month averaging <22°C, but with at least 4 months averaging >10°C. Cfwb x climate types are characterized as having minimum rainfall during winter (February-March) and only one maximum rainfall event that mainly occurs in early summer (June).

Climate data used in this study (i.e., mean air temperature and total amount of rainfall) were obtained from the Croatian Meteorological and Hydrological Service for each year of sampling and analyzed per field site. The distance between the meteorological stations and trapping localities was a maximum distance of 20 km.

From all of the fields investigated soil samples were taken to the depth of a plow layer (30 cm). In each field, five sub-samples (each 300–400 g in weight) were taken, and sub-sampling sites were spaced 30–40 m apart depending on size of field sites). The five sub-samples were then pooled and homogenized and a sub-set of the pooled soil from each site was analyzed. Sediment grain size and chemical properties analyses were conducted at the pedology laboratory of the
Department of Soil Science, Faculty of Agriculture, University of Zagreb, and included the following: percentage of coarse and fine sand, coarse and fine silt, and clay, humus and pH in H2O and KCl.

Soil texture was determined by sieving following standard methods (ISO 11277 2004). Sediment size was classified as: coarse sand (2–0.2 mm); fine sand (0.2–0.063 mm); coarse silt (0.063–0.02 mm); fine silt (0.02–0.002 mm); and clay (<0.002 mm) (Soil Survey Staff 1991). Soil humus (0.3000 g sample weight) was determined by a volumetric titrimetric wet combustion method. For this method soil was placed in Erlenmeyer flask along with 0.1 g Ag2SO4 and 10 ml of 0.4 M K2Cr2O7 solution [19.6 g of potassium dichromate (K2Cr2O7) was dissolved in 500 ml H2O and 500 ml H2SO4 in a volumetric flask of 1 liter]. The mixture was heated for 5 minutes and after it was cooled it was with 150 ml distilled water to a final volume of 300 ml. Titration was carried out by 0.1 M solution of Mohr salt [39.22 g FeSO4 (NH4)2SO4·6H2O was dissolved in 20 ml H2SO4 and 980 ml H2O] with the addition of 2 ml of a mixture of phosphoric acid and sulfuric acid (ratio of 1:1) and two drops of redox indicator (diphenylamine). Equivalence point is appearance of clear dark green solution color.

**Pheromone Trapping.** Csalomon YATLORf funnel traps were used to collect adult *A. brevis*, *A. lineatus*, *A. sputator*, and *A. obscurus* and Csalomon YATLORf3 traps were used to collect *A. ustulatus* (Furlan et al. 2001a). Pheromone vials for each of the five *Agriotes* species were placed singly inside the pheromone traps prior to trap placement. YATLORf funnel traps were set fields just above the soil surface with the funnel bottom buried into the soil. YATLORf3 traps were placed on wooden sticks at a height of 1.5 m. Trapping occurred for *A. brevis*, *A. sputator*, *A. lineatus*, and *A. obscurus* from the 18th to the 32nd weeks of the year, and for *A. ustulatus* from the 23rd to the 32nd weeks of the year. Traps were placed at least 20 m apart and inspected once a week. Pheromone vials were replaced every 6 weeks. During each weekly observation period all adults caught were collected from the traps and counted. Complete pheromone trapping was performed following the manufacturer’s guidelines. Species identification was double checked for *A. brevis* and *A. sputator* which are attracted by the same lure; ~2–3% of the total captures were *A. sputator* individuals as determined using a taxonomic identification key in Klausnitzer (1994).

**Data Analysis.** Adult click beetle population densities at each trapping location was classified according to provisional categories set by Furlan et al. (2001a) as follows: high > 500 adults/trap/season; medium = 50–500 adults/trap/season; low < 50 adults/trap/season; no = no specimens. These limit values were not considered as economic thresholds.

Meteorological data (mean air temperature and the total amount of rainfall), the physical and chemical properties of the soil, and the average number of *Agriotes* spp. individuals were analyzed by a one-way analysis of variance (ANOVA; Gylling Data Management, Inc., USA, ARM 7 GDM software, Revision 7.2.2. 2005). A Tukey’s post hoc test was used to establish climatic differences among the investigated counties and the year of investigation where they occurred.

Exploratory data analysis, using regression tree analyses was done in R 2.30 (R Core Team 2012), applying the package ‘tree’ (Ripley 2012). All variables (number of collected click beetles, mean air temperature, total amount of rainfall, percentage of coarse and fine sand, coarse and fine silt and clay, humus and soil pH in H2O and KCl, crop and precip) were included in the regression tree analysis model. Regression trees are a form of exploratory data analysis that consider which variables contribute to the greatest level of variability explaining the response variables (Zar 2010), in this case the abundance of five species of genus *Agriotes*. A different model and analysis was used and run for each species. The most parsimonious model selected was the model that explained the greatest level of variation within the first split of the regression tree output. Because not all the variables were included in each run of the model (as it is assumed that at least 10 data points are required to complete a statistically valid regression analysis (Zar 2010)), a number of model iterations were employed using different combinations where variables were either added or subtracted. Where a variable was not included in the model (i.e., it did not significantly contribute to explaining as much variability as other variables), it was substituted for another variable. This process continued until the most parsimonious model remained.

**Results**

**Climatic and Edaphic Factors.** Climatic conditions differed among counties and between the investigated years. Significant differences in mean air temperatures occurred as did the total amount of rainfall (Table 1). A detailed description of the regional physical and chemical soil properties are given in Table 2.

**Pheromone Trapping.** In total, 24,506 *Agriotes* individuals were collected of which 1,873 individuals were *A. brevis*, 6,791 individuals were *A. lineatus*, 1,218 individuals were *A. obscurus*, 2,947 individuals were *A. sputator* and 11,677 individuals were *A. ustulatus*.

*Agriotes brevis.* Based on the categories set by Furlan et al. (2001a), in the Koprivnica-Križevci County, populations of *A. brevis* in 2007 and 2008 were classified as ‘low’, while in 2009 population densities were classified as ‘medium’. In the Virovitica-Podravina County the population density was classified as ‘low’ in 2007, but in 2008 and 2009 it was classified as ‘medium’. In the Vukовар-Сirmium County, abundances were consistently ‘low’ from 2007 to 2009. Significant differences in the abundances of *A. brevis* were not observed among the three counties examined, but there were significant differences among the years of investigation (Table 3).

The best predictor for the occurrence of *A. brevis* was the previous crop in all years sampled (2007–2009). The most parsimonious regression tree model predicted that the highest density of *A. brevis* would be found if the previous crop (i.e., precip) was wheat, barley or soybean, with a lower density predicted if corn, sugar beet, white clover, or alfalfa were grown. The highest density of individuals were predicted when soil pH in KCl was between 5.07 and 6.89 (Fig. 2a). Where the previous crop was corn, sugar beet, white clover, or alfalfa and the average temperature was < 11.45°C, the regression tree predicted that a moderate density of individuals would be found. Finally, where average temperature was > 11.45°C and the current crop was sugar beet, barley, or oats, then a lower density of individuals were predicted to be found (Fig. 2b).

*Agriotes lineatus.* In the Koprivnica-Križevci County during 2007 and 2008, populations of *A. lineatus* were classified as ‘medium’, but during 2009 population densities were classified as ‘high’. In the Virovitica-Podravina County population density was classified as ‘low’

<table>
<thead>
<tr>
<th>County</th>
<th>Mean air temperature (°C) ± SD</th>
<th>Total amount of rainfall (mm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koprivnica-Križevci</td>
<td>11.5 ± 0.08c</td>
<td>751.5 ± 53.61a</td>
</tr>
<tr>
<td>Virovitica-Podravina</td>
<td>11.67 ± 0.33b</td>
<td>799.38 ± 80.62a</td>
</tr>
<tr>
<td>Vukовар-Сirmium</td>
<td>13.05 ± 0.05a</td>
<td>665.01 ± 138.27b</td>
</tr>
</tbody>
</table>

*Means followed by the same letter are not significantly different (P > 0.05; Tukey’s HSD).
The abundances of the five adult *Agriotes* species investigated differed according to climatic and edaphic factors and specific environmental variables were identified that can be used to predict their distribution and abundance. Previous studies on the *Agriotes* species in Croatia mainly discuss correlative relationships between wireworm abundance and climate and other environmental factors (physical and chemical soil properties) (Camprag 1997, Maceljski 2002). Further to such studies Staudacher et al. (2013) recently demonstrated that a correlative relationship exists between larval occurrence and edaphic as well as climatic factors (pH, humus, water holding capacity). In contrast there is a great deal of data on the abundance of larvae in fields where previous crops were legumes or other high density planting crops (Camprag 1997, Maceljski 2002), but there is no data on whether a previous crop (i.e., planted the year before larvae are sampled) has any influence on the abundance of adult *Agriotes*. A review of the published literature suggests that click beetles are poor fliers and move only short distances (Camprag 1997, Ester and van Rozen 2005, Maceljski 2002), but there is no data on whether a previous crop (i.e., planted the year before larvae are sampled) has any influence on the abundance of adult *Agriotes*. A review of the published literature suggests that click beetles are poor fliers and move only short distances (Camprag 1997, Ester and van Rozen 2005, Maceljski 2002).
Sufyan et al. (2013) so the majority of individuals caught on pheromone traps have developed from larvae in the same or neighboring fields (Schallhart et al. 2011). Therefore our findings that previous crops significantly impact upon *Agriotes* adult densities are an important one for not only *Agriotes* ecology but also for their management.

*A. brevis*. *A. brevis* abundances in all three counties were at ‘medium’ levels, a result previously reported by Furlan et al. (2001b). Recently, Bažok and Igrc Barčić (2010) showed that abundances in the western counties of Croatia were ‘medium’ to ‘high’. *A. brevis* is considered a major pest of corn and other field crops in Italy (Furlan 1999, Furlan et al. 2000) and is five times more harmful than *A. ustulatus* (Furlan 2011), hence being able to predict their occurrence and levels of abundance is very important for management and control purposes. Furlan (2009) developed a system that predicts wireworm density in the following year and thus determines thresholds based on the number of adults caught in pheromone traps. From the previous author’s work it is suggested that > 300 *A. brevis* adults caught per pheromone trap in 1 year is considered as ‘high’ population abundance. Based on this result it is possible to predict that in the following year one larva/m² of soil will be found (Furlan 2009). However, we used multiple linear regression analyses, to predict occurrence and abundance of *A. brevis* and over a 3-year period found that the previous crop sown was the best predictor of its occurrence and abundance (Fig. 2a and b). Tackenberg et al. (2011) suggested that this species suits colder climates (around 15°C) and Toth (1984) stated that *A. brevis* was more readily found in wetter soils that were rich in humus. Our results confirm higher abundances during periods of lower temperatures; however, we did not find that humus influenced its abundance. Nevertheless, soil pH in KCl was a better predictor of *A. brevis* abundance under Croatian conditions.

*A. lineatus*. The ‘medium’ to ‘high’ densities of *A. lineatus* found in this study generally conform to the results of previous studies conducted in western Croatia (Danon 1960, Maceljski 2002, Bažok 2007, Bažok and Igrc Barčić 2010). Furlan et al. (2001b) reported ‘high’ population densities in eastern Croatia, while our results showed ‘low’ population densities in the same County. In this study, we showed that current crop was the most important factor for predicting the abundance of *A. lineatus*. As the plants of the family Gramineae are known as a suitable food source for adults *A. lineatus* (Toth 1984), it is understandable why a previous crop of wheat was attractive to adults of the species. Our findings are supported by the work of Štrbac (1983) who found that a higher occurrence of *A. lineatus* larvae can be expected in fields if the previous crop was wheat, barley or alfalfa since these cultures are attractants for oviposition. In addition to previous crop, climate variables were also indicated as important in predicting higher densities of the species. However, our results differed to those of Tackenberg et al. (2011) who found that adults were more active at higher temperatures. Although many authors state that this species prefers wetter soils, their findings only relate to the conditions necessary for larval development (Toth 1984; Čamprag 1997;
Maceljski (2002). Our results showed that large soil humus content had a positive impact on population abundance as previously suggested by Staudacher et al. (2013). Ibbotson (1958) showed that an increase in soil pH had a positive impact on species abundance while Staudacher et al. (2013) found the opposite. However, in our study we did not find that soil pH influenced abundance. Rather we found that only soil humus content and average temperatures were important in predicting *A. lineatus* abundance.

*Agriotes obscurus.* Only in 2007 was the abundance of *A. obscurus* classified as ‘medium’ which was similar to the findings of Bazok and Igrc Barčić (2010) who showed that population densities of the same species in central Croatia were ‘low’ to ‘medium’. Furlan et al. (2001b) also found similar results to our study by showing that the population densities of *A. obscurus* in central and eastern Croatia were classified as ‘medium’. Although, Maceljski (2002) found that *A. obscurus* often occurred with *A. lineatus*, we were not able to confirm this in our study.

Previous and current crop did not have a significant impact on predicting the abundance of *A. obscurus*, although Štrbac (1983) found that this species preferred soils where white clover or alfalfa were grown. According to Blackshaw and Hicks (2013), this species can be found with all crops and there was not one single crop that was more important than another in predicting its occurrence. In our study, soil pH in KCl was the most important variable in predicting the abundance of this species. The regression tree results were similar to the results of Ibbotson (1958) who found that its abundance was higher in soils with a lower pH.

*Agriotes sputator.* ‘Low’ to ‘medium’ population densities of *A. sputator* found in this study was similar to those found by Furlan et al. (2001b) and Bazok (2007). Although there were significant differences in average abundance per field, there were no significant differences among counties (Table 3). These results indicated that *A. sputator* was equally represented in all investigated counties and that its abundance
depended more on the year of collection than on the area being investigated.

According to the regression tree results (Fig. 5), the total amount of rainfall was the best predictor for the abundance of this species. The next most important factors for predicting its abundance were current crop [white clover, alfalfa, sugar beet, or barley; confirming the findings of Štrbac (1983) and Čamprag (1997)], and soil pH in KCl. At present there is a lack of published literature and data on the influence that various climatic variables have on the abundance of A. spator making it difficult to compare our results with others.

Agriotes ustulatus. Our findings on the abundance of A. ustulatus were similar (i.e., ‘medium’ to ‘high’) to those reported by previous studies (Štrbac 1983, Furlan et al. 2001b, Maceljksi 2002, Bažok 2007, Bažok and Igre Barčić 2010).

The results of the multiple linear regression analysis indicated that soil pH in KCl was the best predictor of A. ustulatus abundance and that rainfall and soil humus content could also affect its abundance. Our results confirm the findings of Furlan (1996, 1998), that soil moisture is an important factor in the development of the species. However, these results are in contrast to the findings of Toepfer et al. (2007) who showed that soil moisture did not correlate with its density and distribution.

Many studies have been conducted but in just few were established correlation between click beetle abundance and the amount of larvae infection. In Italy Furlan et al. (2001c, 2007) found a correlation existed between A. brevis and A. ustulatus adults caught by pheromones with wireworms found in soil. Pristavko (1988, cit. Čamprag, 1997) found a correlation existed between A. obcurus and A. spator adults caught by pheromones and the abundance of wireworms and the degree of crop damage. Finally, Blackshaw and Vernon (2008) and Blackshaw et al. (2009) found that the pheromone catch of adult A. obcurus is associated with the number of larvae found in the soil. These authors also stated that the number of adults could be used to predict the appearance of larvae and the resulting damage caused. From the research conducted herein, we found that it was possible to identify the factors that have a greater influence on the adult abundance of five Agriotes species under Croatian conditions. Generally, click beetle abundance significantly varies by location; nevertheless the most abundant species were A. ustulatus and A. lineatus. The identified differences in the number and prevalence of species, together with the differences in climatic and edaphic factors enabled us to pinpoint the factors that most affect the number and prevalence of individual Agriotes species in Croatia. We found that humus content and soil pH in KCl were generally the most common predictors of click beetle abundance. Results from this study will contribute to identifying the most common species to each region and based on prevailing climatic and edaphic conditions and consequently further work must be conducted in determining whether a relationship exists between above ground adult abundance and below ground wireworm densities. In this study, we have demonstrated the utility of regression tree in providing a better understanding of how agro-ecological factors influence Agriotes adult population density. These techniques should be considered in future studies to establish a possible correlation between harmful wireworms and adult abundance which would provide sound data for its control.

Supplementary Data

Supplementary data are available at Journal of Insect Science online.

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