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Spread, mass occurrence and damages of *Ophraella communa* LeSage, 1986 on *Ambrosia artemisiifolia* L. in continental Croatia

Širenje, masovna pojava i štete od vrste *Ophraella communa* LeSage, 1986 na vrsti *Ambrosia artemisiifolia* L. u kontinentalnoj Hrvatskoj

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ABSTRACT

Based on targeted faunistic surveys, 34 new occurrences of ragweed leaf beetles (*Ophraella communa*, LeSage) in ten counties of continental Croatia are presented. From mid-July to the end of September 2022, the 34 locations, where ragweed (*Ambrosia artemisiifolia* L.) regularly occurs, were visually inspected. All developmental stages of the insect were found at all locations during July and August, while only adults were found at all locations in September. At 12 locations, ragweed infestation was estimated to be less than 10%. At 10 locations, damage caused by feeding ranged from 10 to 50%, and at seven locations from 50 to 80%. The mass occurrence of ragweed leaf beetles and complete destruction of common ragweed plants in Croatia was recorded at five monitored locations in four counties. A high level of foliar damage indicates that the species has been well established in the monitored area. The ragweed leaf beetle appears to be spreading rapidly over large areas and is capable of effectively controlling invasive common ragweed. Future work should focus on monitoring the spread, establishment, and impact of this species in Croatia and its inclusion in biological ragweed control measures.

Keywords: common ragweed, ragweed leaf beetle, mass occurrence, damage level, biological control

SAŽETAK

Na temelju ciljanih faunističkih istraživanja prikazane su 34 nove pojave ambrozije zlatice (*Ophraella communa*, LeSage) u deset županija kontinentalne Hrvatske. Od sredine srpnja do kraja rujna 2022. vizualno su pregledane 34 lokacije na kojima se ambrozija (*Ambrosia artemisiifolia* L.) redovito pojavljuje. Na svim lokacijama pregledanim u srpnju i kolovozu pronađeni su svi razvojni stadiji kukca, dok su na svim lokacijama pregledanim u rujnu pronađene samo odrasle jedinke. Na 12 lokacija oštećenje ambrozije uzrokovano ishranom ambrozijinom zlaticom procijenjena je na manje od 10%. Na 10 lokaliteta oštećenje uzrokovano ishranom se kretalo od 10 do 50%, a na sedam lokaliteta od 50 do 80%. U Hrvatskoj je, na pet praćenih lokaliteta u četiri županije, zabilježena prva masovna pojava ambrozijine zlatice i potpuno uništenje biljaka ambrozije. Visoka razina oštećenja lišća ukazuje na to da je vrsta već neko vrijeme prisutna na promatranom području. Ambrozijina zlatica se ubrzano širi na velikim površinama i sposobna je vrlo učinkovito suzbiti invazivnu biljnu vrstuambrozije. Budući rad trebao bi biti usmjeren na praćenje širenja, uspostavljanja populacije i utjecaja ove vrste u Hrvatskoj te njezino uključivanje u biološke mjere suzbijanja ambrozije.

Ključne riječi: ambrozija, ambrozijina zlatica, masovna pojava, stupanj oštećenosti, biološko suzbijanje

INTRODUCTION

Common ragweed (*Ambrosia artemisiifolia* L.), a member of the Asteraceae family, is an invasive alien plant in Europe harmful in agricultural production and a source of highly allergenic pollen that causes irritations in 10% of the Croatian (and European) population (Galzina et al., 2010). Common ragweed has been detected in almost all European countries, but in varying densities (Daisie, 2009). It is one of the most important weeds in maize, sugar beets, soybeans, and cereals, where it causes yield losses of up to 80% (Kémives et al., 2006).

Mechanical and chemical control methods are regularly used against common ragweed but are not suitable for all infested locations (Buttenschon et al., 2010). Several species of invertebrates and fungal pathogens are known as potential biocontrol agents. Around 20 fungal pathogens is associated with *Ambrosia* species in Eurasia (Gerber et al., 2011). The biggest effect on ragweed was observed with infection by fungal pathogens: *Phyllachora ambrosiae* and *Plasmopara halstedii* (reported for Hungary: Vajna et al., 2000; Vajna, 2002). Gerber et al. (2011) state that there is almost 40 insect species with ragweed in Eurasia. Most of these insect species cause only moderate damage to ragweed. The only exception is the moth *Ostrinia orientalis* (Lepidoptera: Pyralidae), which was found to significantly reduce ragweed plants (Wan et al., 2003). Among damaging insect species is also *Ophraella communa* LeSage, 1986, the ragweed leaf beetle, which belongs to the order Coleoptera and the family Chrysomelidae (Teshler et al., 2001).

The beetle *O. communa* is a natural enemy of ragweed in Canada and the United States. It was discovered in Europe in 2013 where it was most likely accidentally introduced by air traffic or commercial exchanges at the airport (Müller-Schärer et al., 2014). The first beetles were observed in Italy and Switzerland (Boriani et al., 2013; Müller-Schärer et al., 2014). In the following years, *O. communa* appeared in Slovenia (Devetak et al., 2019), Croatia (Zadravec et al., 2019), Serbia (Petrović-Obradović et al., 2020), Hungary (Horvath and Lukatsi, 2020), and Bosnia and Herzegovina (Vidović et al., 2022).

O. communa is an oligophagous beetle that feeds on plants of the Asteraceae family including tribe Heliantheae, e.g. sunflowers (*Helianthus annuus* L.) and rough cockleburs (*Xanthium strumarium* L.) (Chao et al., 2011), but the main predilection is for common ragweed (Kim and Lee, 2019). Due to the great importance of common ragweed, the occurrence of *O. communa* in Europe has attracted much attention. *O. communa* is a multivoltine beetle whose larvae and adults can completely skeletonize common ragweed leaves, resulting in severe defoliation and pollen reduction, and can even kill common ragweed before flowering (Lommen et al., 2017; Cardarelli et al., 2018). Fertile adult females overwinter in soil debris, eggs are laid in clusters on host plant leaves, and mature larvae spin cocoons on leaves and stems (Bosio et al., 2014).

In recent years, *O. communa* has been increasingly used for biological control of common ragweed (Palmer and Goeden, 1991; Teshler et al., 2000; Zhou et al., 2008; Guo et al., 2011; Zhou et al., 2011). Kiss (2007) even believes that *O. communa* may be one of the most promising candidates for a biological control agent against common ragweed in Europe as well. *O. communa* was found accidentally in Croatia (Zadravec et al., 2019).

According to Zadravec et al. (2019) survey, the beetles were found at ten locations, with the highest density recorded in the Lonjsko Polje Nature Park (Sisak-Moslavina County), near the border with Bosnia and Herzegovina. Reported damage was low to moderate without threatening common ragweed development. Following the first detection of *O. communa* in Croatia in 2018, this study was conducted to verify the establishment and further spread of *O. communa* in continental Croatia and to assess the level of damage to common ragweed plants.

MATERIALS AND METHODS

From mid-July to the end of September 2022, *O. communa* was monitored at 34 locations on the Croatian mainland (in 10 counties). Details of monitored locations are provided in Table 1. The counties surveyed in this study have an area of approximately 25,000 km^2.

Table 1. List of monitored localities with geographical coordinates for *O. communa* in Croatia

No. R.br.	Location Lokacija	County Županija	Coordinates Koordinate
1.	Stupovača	Sisak-Moslavina County	45.5392° N, 16.8403° E
2.	Kutina	Sisak-Moslavina County	45.4628° N,16.7678° E
3.	Đurđevac	Koprivnica-Križevac County	46.0143° N, 17.0203° E
4.	Štaglinec	Koprivnica-Križevac County	46.0805° N, 16.5114° E
5.	Terezino Polje	Virovitica-Podravina County	45.9343° N, 17.4612° E
6.	Pobjenik	Bjelovar-Bilogora County	45.4232° N ,16.3655° E
7.	Suhaja	Bjelovar-Bilogora County	45.4325° N, 16.3742° E
8.	Općevac	Bjelovar-Bilogora County	45.7277° N, 16.5568° E
9.	Bojana	Bjelovar-Bilogora County	45.7561° N, 16.6815° E
10.	Krivaja	Bjelovar-Bilogora County	45.7126° N, 16.8202° E
11.	Velika Trnovitica	Bjelovar-Bilogora County	45.6861° N, 16.9533° E
12.	Hercegovac	Bjelovar-Bilogora County	45.6560° N, 17.0105° E
13.	Dežanovac	Bjelovar-Bilogora County	45.5691° N, 17.0861° E
14.	Veliki Zdenci	Bjelovar-Bilogora County	45.6645° N, 17.0910° E
15.	Globočec	Krapina-Zagorje County	46.0040° N, 16.0904° E
16.	Donje Orešje	Zagreb County	45.9974° N, 16.2430° E
17.	Staro Čiće	Zagreb County	45.6968° N, 16.1058° E
18.	Velika Mlaka	Zagreb County	45.7354° N, 16.0486° E
19.	Petrovina Turopoljska	Zagreb County	45.6894° N, 16.0177° E
20.	Tomašanci	Brod-Posavina County	45.3992° N,18.4122° E
21.	Osijek	Brod-Posavina County	45.5185° N,18.7820° E
22.	Donji Andrijevci	Brod-Posavina County	45.1831° N,18.3277° E
23.	Opatovac	Brod-Posavina County	45.3143° N,17.4374° E
24.	Cernik	Brod-Posavina County	45.2787° N,17.4027° E
25.	Slavonski Brod	Brod-Posavina County	45.1769° N,18.0464° E
26.	Stari Slatnik	Brod-Posavina County	45.1872° N,17.8627° E
27.	Vukovar	Vukovar-Syrmia County	45.3624° N,18.9738° E
28.	Nuštar	Vukovar-Syrmia County	45.3260° N,18.8284° E
29.	Vinkovci	Vukovar-Syrmia County	45.2200° N,18.7856° E
30.	Gradište	Vukovar-Syrmia County	45.1520° N,18.7336° E
31.	Županja	Vukovar-Syrmia County	45.1047° N,18.7110° E
32.	Blacko	Požega-Slavonia County	45.3127° N,17.7800° E
33.	Završje	Požega-Slavonia County	45.3275° N,17.6287° E
34.	Bilice	Požega-Slavonia County	45.2424° N,17.8391° E

monitored in July monitored in August monitored in September

The locations were selected because they were known to already have large populations of common ragweed. Single random visual observation and direct sampling method were used to assess beetle populations at all locations. The monitored area per location was 1 m^2, in which the presence (and developmental stages) of *O. communna* and the readings of damage on ragweed plants were recorded. The number of plants within monitored square meter depended on common ragweed population and varied from four to six plants. The collected specimens were placed in entomological cages and taken to Entomological Laboratory of the Faculty of Agriculture, where they were identified, counted, and preserved in 70% EtOH. The species *O. communa* was identified using SMARTER (2014) and Warchałowski (2010). To determine the percentage of damage to common ragweed plants, all plants were examined in an area of 1 m^2. The percentage of damage was calculated based on the destroyed leaf area of all plants present on the monitored area.

RESULTS AND DISCUSSION

The results of presented study are concrete proof of establishment and spread of *O. communa* in continental Croatia in 2022. In our study, *O. communa* was found in 10 different counties at a total of 34 locations. The results of this study, including beetle stages recorded at each location and common ragweed damage assessed, are presented in Table 2. *Ophrella communa* was found quickly, within one minute of visual inspection of common ragweed at all monitored locations in mainland Croatia. No other insects were observed on ragweed plants during the whole study.

All developmental stages of *O. communa* were found at locations monitored in July and August (Figure 1-4). Adult insects were found on all parts of the common ragweed plant. The yellowish eggs were laid in clusters on the upper and lower surfaces of the leaves. The number of eggs per cluster varied from a few to several dozen. Pupae were found on both damaged and undamaged branches. The number of individuals at each life stage varied from plant to plant. At the location Stupovača with more than 80% damaged common ragweed plants, an average of 53 adult ragweed leaf beetles, 71 larvae, 23 pupae, and three egg clusters were found in July. All developmental stages of *O. communa* occurred on common ragweed leaves during this observation period (mid-summer), confirming the studies of Dernovici et al. (2006) and Zhou et al. (2010). At other locations with total common ragweed devastation (> 80%), only adults were found (10-37 per common ragweed plant), but these locations were monitored in September.

Observed common ragweed plant damage was less than 10% at most locations monitored in July. Only at location Stupovača (Sisak-Moslavina County) monitored during the summer months, common ragweed plant damage was estimated to exceed 80%. This location is in the same county where *O. communa* was first found in 2018 (Zadravec et al., 2019). Zadravec et al. (2019) reported on the beginning of invasion, which apparently continues successfully in this area. Our result is a confirmation of the successful overwintering and population development of this species on the Croatian mainland. At other locations monitored in July and August, common ragweed plant damage was mostly very low (< 10%), but population densities were high, indicating that the species had migrated some time ago and the population was already established. In September, we expanded our monitoring area to other locations and to counties in eastern continental Croatia, where extremely high levels of common ragweed plant damage were recorded. Like monitoring results in August, we also recorded high levels of common ragweed plant damage in Sisak-Moslavina County in September. In the eastern counties, the extent of damage varied from very low (10%) to extremely high (100%) (Figures 5-8).

Damage to common ragweed plants by adults and larvae of *O. communa* was massive in several locations on the Croatian mainland. By September, common ragweed plants were completely defoliated and reduced to brown stumps without leaves or flowers (Figure 7). This confirms the study by Bosio et al. (2014), where complete defoliation was observed in the province of Novara in Italy. Although the same authors report about overlapping of

Table 2. *O. communa* developmental stages and assessed damage on common ragweed plants on all monitored locations in continental Croatia

	Developmental stage Razvojni stadij				Common ragweed damage Oštećenje na ambroziji (%)			
Location Lokacija	Eggs Jaja	Larvae Ličinke	Pupae Kukuljice	Adults Odrasli	<10	10-50	50-80	80-100
Stupovača	+	+	+	+				+
Kutina				+				+
Đurđevac	+	+			+			
Štaglinec	+				+			
Terezino Polje	+			+	+			
Pobjenik	+	+	+	+	+			
Suhaja	+	+	+	+	+			
Općevac	+	+	+	+	+			
Bojana	+	+	+	+	+			
Krivaja	+	+	+	+	+			
Velika Trnovitica	+	+	+	+		+		
Hercegovac	+	+	+	+		+		
Dežanovac				+		+		
Veliki Zdenci				+		+		
Globočec	+			+	+			
Donje Orešje	+	+		+		+		
Staro Čiće	+	+	+	+		+		
Velika Mlaka	+	+	+	+		+		
Petrovina Turopoljska	+			+	+			
Tomašanci				+			+	
Osijek				+		+		
Donji Andrijevci				+				+
Opatovac				+			+	
Cernik				+			+	
Slavonski Brod				+	+			
Stari Slatnik				+		+		
Vukovar				+		+		
Nuštar				+			+	
Vinkovci				+	+			

Continued. Table 2.

Location Lokacija	Developmental stage Razvojni stadij				Common ragweed damage Oštećenje na ambroziji (%)			
	Eggs Jaja	Larvae Ličinke	Pupae Kukuljice	Adults Odrasli	<10	10-50	50-80	80-100
Gradište				+			+	
Županja				+				+
Blacko				+				+
Završje				+			+	
Bilice				+			+	

monitored in July monitored in August monitored in September

Figure 1. Ragweed leaf beetle eggs (Photo: Ž. Oštrkapa Međurečan)

Figure 2. Ragweed leaf beetle larvae (Photo: D. Lemic)

Figure 3. Ragweed leaf beetle pupae (Photo: D. Lemic)

Figure 4. Ragweed leaf beetle adults (Photo: H. Viric Gasparic)

Figure 5. Damage of 10% on common ragweed plant (Photo: D. Lemic)

Figure 6. Damage from 30 to 50% on common ragweed plant (Photo: D. Lemic)

generations in September, this situation was not observed in our study. At all locations observed, only adults were present on common ragweed plants in September. Bosio et al. (2014) recorded more than 250 adults on a single plant (60 cm high), while in Croatia only 53 adults were counted on a single plant. Despite the lower number of individuals per plant, the damage to common ragweed was total. The feeding activity, resulting in complete and intense skeletonization of the leaves, stops the growth of the plants and leads to their withering (Figure 8). This extremely intense attack on common ragweed stops flower and pollen production and leads to desiccation of the anthers (Bosio et al., 2014). In Italy, only adults were recorded in October, and Bosio et al. (2014) suggest that they are entering the overwintering stage. We observed only adults in September, which may indicate earlier preparation for overwintering under conditions prevailing in Croatia. In our study same as in Bosio et al. (2014) and Zhu et al. (2012), no eggs were found in September, confirming that adult females enter diapause when the photoperiod shifts from long to short days.

Figure 7. Damage from 50 to 80% on common ragweed plant (Photo: D. Lemic)

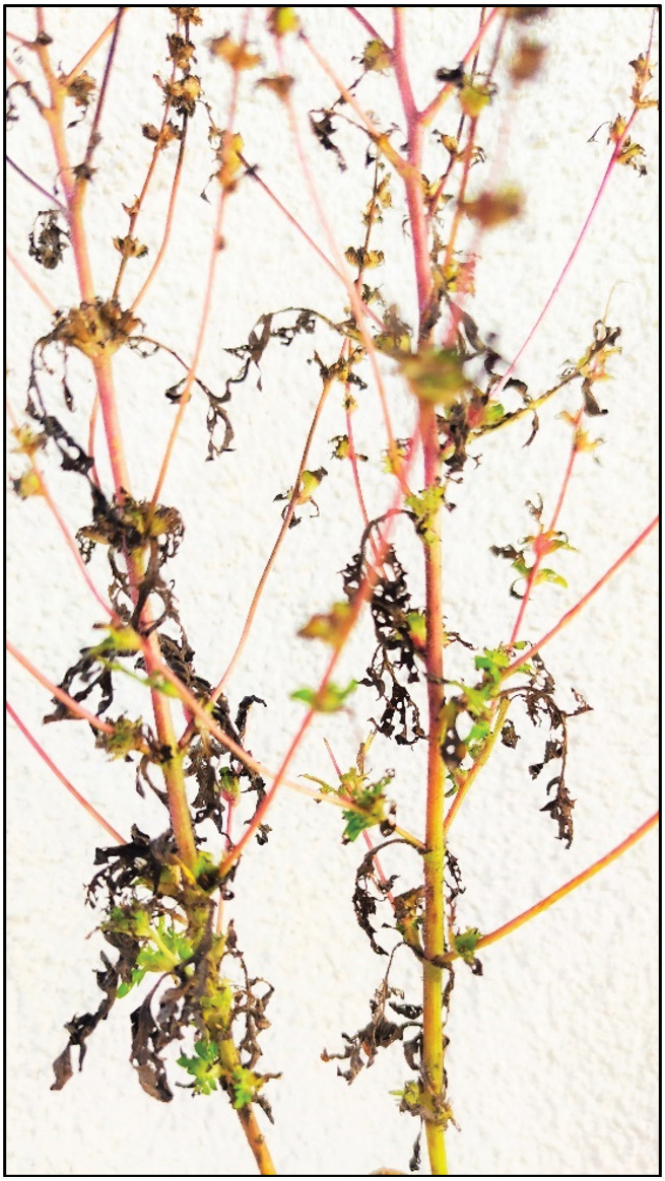

Figure 8. Damage of 100% on common ragweed plant (Photo: D. Lemic)

In this study, *O. communa* was detected in 34 localities in 10 counties covering almost the entire continental Croatia. In this area, the beetle was detected on common ragweed plants growing in urban areas, in ruderal areas or along roadsides, in cultivated or abandoned fields, with no differences in abundance or damage to common ragweed plants. The counties surveyed in this study have an area of approximately 25,000 km^2, and locations were randomly selected to cover the main areas of high common ragweed infestation (Figure 9). Zandigiacomo et al. (2020) recorded *O. communa* in nortwestern Croatia (Primorje-Gorski Kotar County) at four locations, but with very low levels of common ragweed damage. However, that study increases the area infested by *O. communa* in Croatia. Within about four years after the first records in Italy (Boriani et al., 2013), the beetle reached the western areas of Slovenia and the northwestern areas of Croatia (Zadravec et al., 2019), which are more than 400 km away from the presumed location of its introduction into Europe at Milan airport (Müller-Schärer et al., 2014).

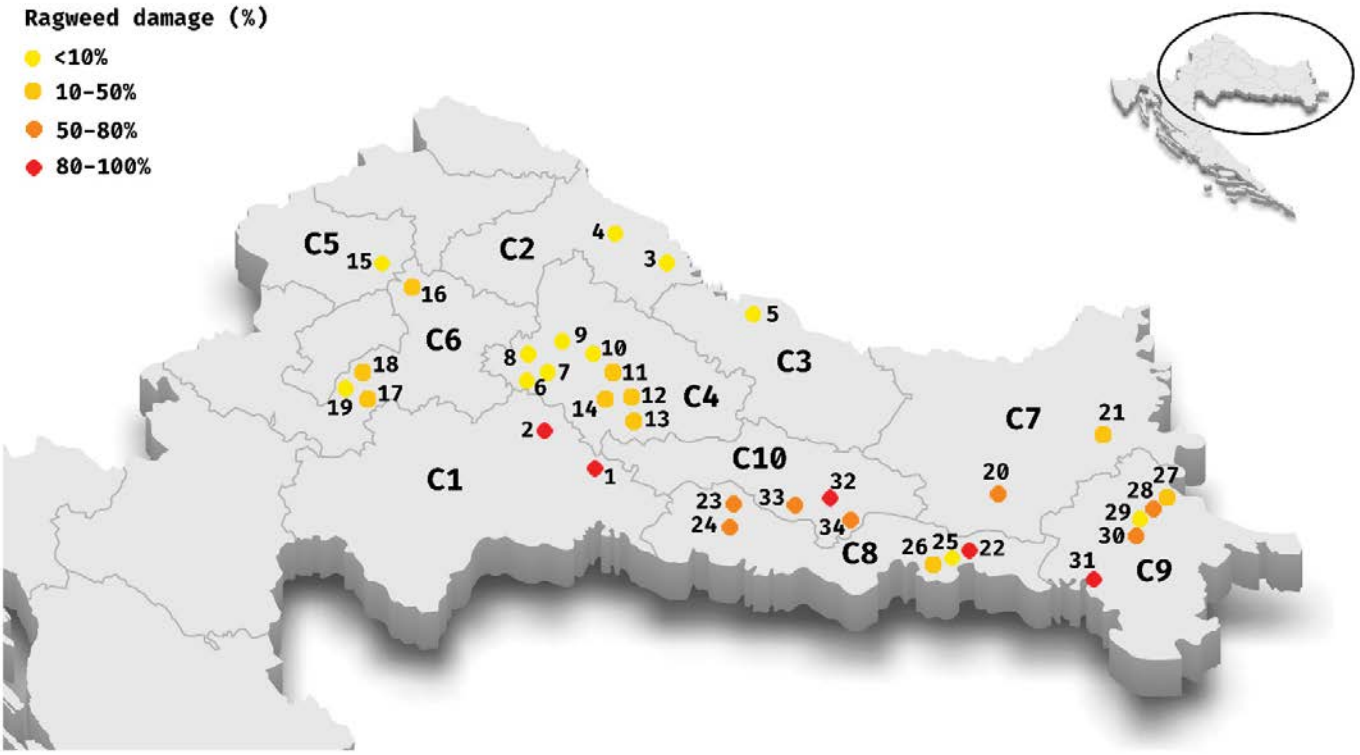

Figure 9. *Ophrella communa* invaded areas in continental Croatia monitored in 2022 with damage (%) on common ragweed plants (location details are shown in Table 1)

Four years after the first records in Croatia (Zadravec et al., 2019) in 2018, when the beetle was detected in central Croatia, *O. communa* spread throughout the continental part of the country and started to cause major damage to common ragweed. Thus, the species has demonstrated a high potential for dispersal. It can spread more than 70 km per year by flight (Kim and Lee, 2019), but an even greater rate of spread can be assumed due to human transport. These data are consistent with observations of the rapid dispersal of *O. communa* at the beginning of its colonization of Japan (Zandigiacomo et al., 2020 cit. Moriya and Shiyake, 2001). Further observations in the northern and southern parts of Croatia, where the species has not yet been found, will provide additional information on the colonization pattern of this potential biocontrol agent of common ragweed (Kiss, 2007; Keszthelyi et al., 2022).

CONCLUSION

The species *O. communa* was first discovered in Croatia in 2018. Only four years later, in 2022, it was detected in the entire continental territory of Croatia. The very rapid and surprising spread of the species in both urban and rural areas occurred in a total of 10 monitored counties, at 34 locations. Damage of common ragweed between 50 and 80% was detected in seven monitored locations. The first mass emergence and 100% destruction of common ragweed plants was recorded at five monitored locations (in four counties throughout continental Croatia). The beetle appears to spread and adapt very quickly over large areas and is very capable of effectively controlling invasive common ragweed.

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