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POSSIBILITIES FOR BIOLOGICAL CONTROL OF VELVETLEAF (*Abutilon theophrasti* Medik.) WITH PHYTOPHAGOUS INSECTS

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Velvetleaf (*Abutilon theophrasti* Medik.) creates a serious weed problem in farmland, in Croatia especially in maize, soybean, sugar beet and sunflowercrops, but worldwide as well. Because of early-season control escape, and the discovery of atrazine-resistant populations of velvetleaf, investigation of insects as potential biological agents for velvetleaf control was conducted in Croatia from 1995 to 2000. In 24 Croatian localities a total of 15 insect species associated with velvetleaf were recorded. A total of 10 insect species: *Carpocoris purpureipennis* De Geer, 1773, *Graphosoma lineatum italicum* Müler, 1766, *Lygeaeus saxatilis* Scopoli, 1763, *Pyrrhocoris apterus* Linnaeus, 1758, *Rhopalosiphum padi* Linnaeus, 1758, *Macrosiphum euphorbiae* Thomas, 1878, *Podagrica menetriesi* Falderman 1837, *Psylliodes brisouti* Bedel, 1898, *Carcharodus alceae* Esper, 1780 and *Autographa gamma* Linnaeus, 1758, were recorded on velvetleaf for the first time. The insect species *Pyrrhocoris apterus*, *Carpocoris purpureipennis*, *Graphosoma lineatum italicum*, *Lygaeus saxatilis* and *Psylliodes brisouti* were recorded on velvetleaf as accidentals and no signs of their feeding on velvetleaf were registered. Well known pest species *Aphis fabae* Scopoli, 1763, *Rhopalosiphum padi*, *Macrosiphum euphorbiae*, *Autographa gamma* and *Ostrinia nubillalis* Hübner, 1796 were recorded on velvetleaf rarely, but the feeding of the mentioned Lepidoptera species was recorded during field observations. Frequently collected insect species *Liorhyssus hyalinus* Fabricius, 1794 f. *hyalina*, *pallida* and *rubricata*, *Crocidosema plebejana* Zeller, 1847 and *Heliothis armigera* Hübner, 1808 had

a great impact on seed survival, while *Podagrica menetriesi* and *Carcharodus alceae* reduced the leaf area of velvetleaf significantly.

***Abutilon theophrasti* - biological weed control - Croatia - phytophagous insect fauna - velvetleaf**

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Europski mračnjak (*Abutilon theophrasti* Medik.) predstavlja značajan problem kao korov u proizvodnji kukuruza, soje, šećerne repe i suncokreta u Republici Hrvatskoj, ali i u svijetu. Zbog neuspjeha u rano-sezonskom suzbijanju ovoga korova i utvrđene rezistentnosti na atrazin, istraživanje mogućnosti biološkog suzbijanja ovoga korova pomoću kukaca provedeno je u periodu od 1995. do 2000.g. U 24 lokaliteta na prostoru Republike Hrvatske na ovoj korovnoj vrsti utvrđeno je ukupno 15 vrsta kukaca. Ukupno 10 vrsta kukaca: *Carpocoris purpureipennis* De Geer, 1773, *Graphosoma lineatum italicum* Müler, 1766, *Lygeaeus saxatilis* Scopoli, 1763, *Pyrrhocoris apterus* Linnaeus, 1758, *Rhopalosiphum padi* Linnaeus, 1758, *Macrosiphum euphorbiae* Thomas, 1878, *Podagrica menetriesi* Falderman 1837, *Psylliodes brisouti* Bedel, 1898, *Carcharodus alceae* Esper, 1780 i *Autographa gamma* Linnaeus, 1758 utvrđeno je na europskom mračnjaku po prvi puta. Vrste kukaca *Pyrrhocoris apterus*, *Carpocoris purpureipennis*, *Graphosoma lineatum italicum*, *Lygaeus saxatilis* i *Psylliodes brisouti* utvrđene su na ovom korovu kao slučajna entomofauna, a njihova ishrana na europskom mračnjaku nije utvrđena. Poznate štetne vrste *Aphis fabae* Scopoli, 1763, *Rhopalosiphum padi*, *Macrosiphum euphorbiae*, *Autographa gamma* i *Ostrinia nubillalis* Hübner, 1796 na biljkama europskog mračnjaka utvrđene su rijetko, no ishrana navedenih vrsta reda Lepidoptera utvrđena je prilikom provođenja vizualnih pregleda ovoga korova u poljskim uvjetima. Često prikupljane vrste kukaca *Liorhyssus hyalinus* Fabricius, 1794 f. *hyalina*, *pallida* and *rubricata*, *Crocidosema plebejana* Zeller, 1847 i *Heliothis armigera* Hübner, 1808 imaju značajan utjecaj na klijavost sjemena ovoga korova, dok vrste *Podagrica menetriesi* i *Carcharodus alceae* značajno oštećuju I reduciraju lisnu površinu europskog mračnjaka.

***Abutilon theophrasti*, biološko suzbijanje korova, Republika Hrvatska, fitofagna entomofauna, europski mračnjak**

Introduction

Velvetleaf, *Abutilon theophrasti* Medik. is one of the most troublesome and economically important weeds in field crops (maize, soybean, sugar beet, sunflower, cotton) and vegetable fields worldwide. Maize yield reductions from 16 to 93% caused by velvetleaf were reported in the USA (Behrens & Lee, 1966 cit. Spencer, 1984), while 51-91% yield loss was reported in Wisconsin (Sterling & Putnam, 1987 cit. Warwick & Black, 1988). Velvetleaf is a tough, competitive weed in soybeans. An infestation of 5 plants/m^2 caused an average yield reduction of 25% over 2 years, while 40 plants/m^2 caused a yield reduction of 57% (Spencer, 1984). Yield losses recorded in sugar beet ranged between 14 and 30% with 6-24 velvetleaf plants in a 30 m long row (Schweizer & Bridge, 1982 cit. Lesnik, 1999).

Velvetleaf often escapes early-season weed control, especially when soil-applied herbicides fail to perform satisfactorily. This weed often emerges late during the growing season and, if not controlled, will not only compete with crop plants for light, moisture and nutrients but also produce viable seed for addition to the soil seed bank. The average number of seeds is in the 35-45 per capsule range, with 70-199 mature capsules per plant and seed production per plant ranges between 700 and 17000 seeds (Winter, 1960 cit. Warwick & Black, 1988; Chandler & Dale, 1974 cit. Warwick & Black, 1988; Shaw et al., 1974 cit. Warwick & Black, 1988; Hartgerink & Bazzaz, 1984 cit. Warwick & Black, 1988; Anderson et al., 1985 cit. Warwick & Black, 1988; Brown, 1985 cit. Warwick & Black, 1988; Warwick & Black, 1986 cit. Warwick & Black, 1988). Velvetleaf seeds are known to remain viable for up to 50 years when stored dry or in the soil (Warwick & Black, 1988). Selective postemergence herbicides can be used to eliminate velvetleaf escaping early-season control, but the expense of the chemicals and application technology required and potential injury to the crop may not be justified because escapes often do not significantly reduce crop yields (Biniak et al., 1986).

An alternative strategy would be to use biological control agents that effectively prevent seed production and/or reduce seed viability (Kremer & Spencer, 1989). Any kind of damage caused to velvetleaf plants by biological control agents would help in the reduction of the competitive abilities of this weed. According to literature data, 32 insect species have been discovered that might be used to provide effective biological control of velvetleaf (Table 1).

Insect species that have been investigated in more detail are the seed-feeding species: *Helicoverpa zea* Boddie, 1850, *Heliothis virescens* Fabricius, 1777, *Liorhyssus hyalinus*, *Niesthrea louisianica* Sailer, 1961 and *Altheus folkersti* Kingsolver, 1989. Seed-associated fungus species that have been tested as potential biological control agents are: *Fusarium lateritium* (Nees) (Walker, 1981 cit. Warwick & Black, 1988; Boyette & Walker, 1985 cit. Warwick & Black, 1988; Boyette & Walker, 1986 cit. Warwick & Black, 1988; Watson, 1993) and *Myrothecium verrucaria* (Albertini & Schwein). All other insect (27 species, see Table 1), fungus (54 species), bacterial (14 species) or nematode (4 species) species mentioned in the literature were described as associated with velvetleaf and but have not been investigated in detail.

The only one fungus species, *Colletotrichum coccodes* (Wallr.), was applied in the biological control of velvetleaf by the augmentative method (Julien & Griffiths, 1999).

Velvetleaf is one of the most important weeds in row crops in Croatia as well. Preemergence and postemergence herbicides are used to suppress it, but a herbicide-resistant biotype of velvetleaf has been found, and alternative and complementary measures of control are needed. The same problems, with estimated annual control costs of 343 million dollars, are found in maize and soybean in the US. This indicates the major impact of this weed on the U.S. agricultural economy (Spencer, 1984) and led to the joint project US–Croatia JF-206. The main goal of this project, conducted from 1995 to 2000 in Croatia, was a field survey for phytophagous insects associated with velvetleaf that have the potential to become effective biological control agents.

Materials and methods

Surveys for insects on velvetleaf plants were conducted from 1995 to 2000 in 9 Croatian counties: Vukovarsko-srijemska, Osjecko-baranjska, Viroviticko-podravska, Pozesko-slavonska, Brodsko-posavska, Bjelovarsko-bilogorska, Sisacko-moslavacka, Zagrebacka and Grad Zagreb. A total of 24 localities in continental region of Croatia (Magadenovac, Cepin, Grabovac, Knezevo, Nustar, Vinkovci, Stari Mikanovci, Vrpolje, Otok, Komletinci, Bosnjaci, Posavski Podgajci, Drenovci, Tovarnik, Kutjevo, Nova Gradiska, Gradina, Dragicevac, Popovaca, Hercegovac, Grabrov Potok, Lupoglav, Ivanic Grad and Zagreb) were visited from April to November at least two times monthly. Insects were collected

from velvetleaf plants in maize, soybean, sugar beet, oilseed rape and sunflower or from ruderal sites.

Insects were collected from leaves, branched stems, flowers, flower bulbs and fruit-seed capsules, during field observation of plants and plant dissection. Collection of small insects was conducting by entomological aspirator, which consisted of a plastic tube and two plastic pipes which were inserted in the lid. One pipe had a plastic mesh at the end of the tube, to prevent suction of small insects from tube to mouth. Other insect species were collected in plastic or wooden cages, if possible with the plant parts on which they were found. Because velvetleaf is erect and typically grows tall (1-2.5 m or taller) insects could not be collected using the usual sweeping method. Dissected stems of velvetleaf in which the caterpillars of some species were found, were fixed again and transported to the greenhouse.

When only a single or a few individuals of a species were found, the association with velvetleaf was considered accidental. Only species occurring more frequently or sampled at several localities were considered to be associated with the target weed. The degree of association could only be determined by rearing. Therefore, collected insects were transported to the greenhouse where immature stages were reared to adults (on leaves, stems or seed capsules of velvetleaf) and forwarded for identification to entomologists in Croatia and Germany.

Feeding tests were made with species frequently collected. In greenhouse conditions, collected insects were tested for feeding on velvetleaf, and for some insect species also on some other plant species, in “no choice” feeding tests. In feeding tests, plants were chosen by the centrifugal philogenetic method (Wapshere, 1971; Wapshere, 1974), which also proposes the testing of insect feeding on some important cultivated plant species. For feeding tests, whole velvetleaf plants were taken from the fields and transported to the greenhouse where they were transplanted into pots. In tests in which insects had to be reared on seed capsules, the seed capsules were cut with part of the branches and brought into the greenhouse. Fresh leaves for the feeding tests were cut from plant branches in the field or in the greenhouse and were put in tubes filled with water. During feeding tests insects were isolated on plant parts in plastic or wooden cages covered with a light cover. Observations and measuring of insect development and feeding were conducted daily, and fresh plant parts were added as necessary.

Table 1. Insect species associated with velvetleaf plants worldwide

Species	Country	Reference
Thysanoptera: Thripidae		
Thrips tabaci Lindeman, 1889	Slovenia	Lesnik, 1999
Tusothrips aureus Mouleon	USA	Zheng et al., 2004
Heteroptera: Miridae		
Calocoris norvegicus Gmelin, 1790	Slovenia	Lesnik, 1999
Lygus pratensis Linnaeus, 1758	Slovenia	Lesnik, 1999
Lygus rugulipennis Poppius, 1911	Slovenia	Lesnik, 1999
Lygus campestris Linnaeus, 1758	Slovenia	Lesnik, 1999
Dicyphus errans Wolf J.F, 1804	Italy	Pemberton & Hoover, 1980
Heteroptera: Pyrrhocoridae		
Dysdercus cingulatus Fabricius, 1775	USA	Zheng et al., 2004
Heteroptera: Rhopalidae		
Liorhyssus hyalinus Fabricius, 1794	USA	Gibb, 1991; Zheng et al., 2004
Niesthrea louisianica Sailer, 1961	USA	Gibb, 1991
Heteroptera: Lygaeidae		
Oxycarenus hyalipennis Costa, 1843	Italy	Pemberton & Hoover, 1980
Homoptera: Cicadidae *Empoasca flavescens* Fabricius, 1794	Slovenia	Lesnik, 1999
Homoptera: Aleyrodidae		
Trialeurodes vaporariorum Westwood, 1856	USA	Headrik et al., 1997 cit. Lesnik, 1999
Bemisia tabaci Gennadius, 1889	USA	Headrik et al., 1997 cit. Lesnik, 1999
Bemisia argentifolii Westwood et al. 1994	USA	Headrik et al., 1997 cit. Lesnik, 1999
Homoptera: Aphididae		
Aphis fabae Scopoli, 1763	Slovenia	Lesnik, 1999
Aphis gossypii Glover, 1877	Slovenia	Lesnik, 1999
Homoptera: Pseudococcidae		
Pseudococcus maritimus Ehrhorn, 1900	USA	Zheng et al., 2004
Coleoptera: Bruchidae		
Altheus folkersti Kingslover, 1989	USA	Gibb, 1991

Table 1. Continued

Species	Country	Reference
Altheus hibisci Olivier, 1795	USA	Kingslover et al., 1989
Altheus steineri Kingslover, 1989	USA	Kingslover et al., 1989
Coleoptera: Chrysomelidae		
Systena frontalis Fabricius, 1801	USA	Mitich, 1991
Lepidoptera: Noctuidae		
Actontia malvae Esper	USA	Zheng et al., 2004
Anomis flava Fabricius, 1775	USA	Zheng et al., 2004
Helicoverpa zea Boddie, 1850	USA	Gibb, 1991; Headrik et al., 1997 cit. Lesnik, 1999
Heliothis virescens Fabricius, 1777	USA	Gibb, 1991; Headrik et al., 1997 cit. Lesnik, 1999
Heliothis armigera Hübner, 1808	USA	Zheng et al., 2004
Lepidoptera: Nymphalidae		
Hypolimnas missipus Linnaeus, 1764	USA	Zheng et al., 2004
Lepidoptera: Pyralidae		
Ostrinia nubillalis Hübner, 1796	Slovenia	Lesnik, 1999
Lepidoptera: Tortricidae		
Crocidosema plebeyana Zeller, 1847	Italy	Pemberton & Hoover, 1980
Diptera: Sarcophagidae		
Sarcophaga sp. Meigen, 1826	Greece	Pemberton & Hoover, 1980
Diptera: Tachinidae		
Exorista larvarum Linnaeus, 1758	Greece	Pemberton & Hoover, 1980

Results

15 insect species associated with velvetleaf were recorded in faunistic investigation conducted in a total of 24 Croatian localities (Table 2). A total of 10 new insect species: *Carpocoris purpureipennis*, *Graphosoma lineatum italicum*, *Lygeaeus saxatilis*, *Pyrrhocoris apterus*, *Rhopalosiphum padi*, *Macrosiphum euphorbiae*, *Podagrica menetriesi*, *Psylliodes brisouti*, *Carcharodus alceae* and *Autographa gamma*, which were not recorded in literature data, were identified.

Table 2. Insect species associated with velvetleaf plants in Croatia

Species	Type of association	Locality
Heteroptera: Lygaeidae		
Lygaeus saxatilis Scopoli, 1763	Seed capsules[a]	Vinkovci, Lupoglav
Heteroptera: Pentatomidae		
Carpocoris purpureipennis De Geer, 1773	Seed capsules[a]	Lupoglav
Graphosoma lineatum italicum Müler, 1766	Seed capsules[a]	Lupoglav
Heteroptera: Pyrrhocoridae		
Pyrrhocoris apterus Linnaeus, 1758	Seed capsules, stem, leaves[a]	Stari Mikanovci
Heteroptera: Rhopalidae		
Liorhyssus hyalinus Fabricius, 1794 f. *hyallina, pallida, rubricata*	Seed capsules[b]	Lupoglav, Otok, Vinkovci, Stari Mikanovci
Homoptera: Aphididae		
Aphis fabae Scopoli, 1763	Stem, leaves[a]	Zagreb
Rhopalosiphum padi Linnaeus, 1758	Stem, leaves[a]	Lupoglav
Macrosiphum euphorbiae Thomas, 1878	Stem, leaves[a]	Zupanja
Coleoptera: Chrysomelidae		
Podagrica menetriesi Falderman, 1837	Leaves[b]	Stari Mikanovci, Otok
Psylliodes brisouti Bedel, 1898	Leaves[a]	Stari Mikanovci
Lepidoptera: Hesperiidae		
Carcharodus alceae Esper, 1780	Leaves[b]	24 localities[c]
Lepidoptera: Noctuidae		
Heliothis armigera Hübner, 1808	Seed capsules, flower buds[b]	Otok, Lupoglav, Tovarnik
Autographa gamma Linnaeus, 1758	Leaves[b]	Cepin
Lepidoptera: Pyralidae		
Ostrinia nubillalis Hübner, 1796	Stem, branches[b]	Lupoglav, Stari Mikanovci, Vrpolje
Lepidoptera: Tortricidae		
Crocidosema plebejana Zeller, 1847	Stem, branches, flower bulbs, seed capsules[b]	Stari Mikanovci, Otok, Vrpolje, Lupoglav, Grabov Potok, Popovaca

[a] feeding on velvetleaf was not confirmed
[b] feeding on velvetleaf was confirmed
[c] Bosnjaci, Cepin, Dragicevac, Drenovci, Gradina, Grabovac, Grabrov Potok, Hercegovac, Ivanic Grad, Knezevo, Komletinci, Kutjevo, Lupoglav, Magadenovac, Nova Gradiska, Nustar, Otok, Popovaca, Posavski Podgajci, Stari Mikanovci, Tovarnik, Vinkovci, Vrpolje, Zagreb

The insect species most frequently associated with velvetleaf were: *Liorhyssus hyalinus*, *Podagrica menetriesi*, *Carcharodus alceae*, *Crocidosema plebejana* and *Heliothis armigera*, and their feeding on velvetleaf was detected in both field conditions and greenhouse feeding tests. These species were investigated in more detail, and a description of their impact on velvetleaf plants will be presented.

Heteroptera

In field observations of velvetleaf a total of 5 Heteroptera species were identified. Bug species (adults) *Graphosoma lineatum italicum* (2 specimens), *Carpocoris purpureipennis* (6 specimens) both found in the locality Lupoglav in October 1998, *Lygaeus saxatilis* (2 specimens) found in the localities Vinkovci and Lupoglav in October 1998 and *Pyrrhocoris apterus* found in the locality Stari Mikanovci in 2000 were tested for feeding on velvetleaf seed and in the case of some of the species on leaves and stems in greenhouse. No signs of feeding were recorded in a few days, so those species are considered to be accidentals on velvetleaf plants.

Liorhyssus hyalinus Fabricius, 1794, f. *hyalina*, *pallida*, *rubricata*

Bug species *Liorhyssus hyalinus* f. *hyalina*, *pallida*, *rubricata* was collected in the localities Lupoglav, Otok, Vinkovci and Stari Mikanovci. In the literature *Lyorhyssus* sp. was recorded on plants from the genera *Mesembryanthemum* L, *Sonchus* L, *Helianthus* L. and *Zea* L. (Stichel, 1955), as well as on velvetleaf (Gibb, 1991; Zheng et al., 2004).

Specimens of *Liorhyssus hyalinus* were collected on velvetleaf plants from August to the middle of November 2000. In that period, numerous velvetleaf seed capsules (immature and mature) and a great number of all development stages of *L. hyallinus* (eggs, larvae and adults at the same time) on seed capsules were present. Bugs were present in a group of 10 or more specimens, mostly on newly-produced seed capsules, but also on mature capsules.

Adults of *Liorhyssus hyalinus* were collected by entomological aspirator, as well as together with the capsules on which they were found, and were transported in greenhouse.

In the greenhouse, feeding of this species was tested only on velvetleaf plants. Bugs, 15 specimens, were isolated on seed capsules of plants, which were planted in pots. Copulation of bugs was recorded, so eggs and later larvae and adults were detected. In greenhouse conditions, the first copulation of bugs

was recorded in August (August 23rd) 2000, and 5 days later (August 28th) the oviposition started. Copulation was also recorded in September (September 12th-15th) 2000 and oviposition was recorded on September 19th 2000. The larvae of different development stages were also recorded in field observations in August, September, October and November. In the greenhouse, in November 2000, when temperatures were about 10°C, the death of bugs was recorded.

These data indicate the relatively easy rearing of this species in laboratory conditions, which could be an advantage if this species were used in the biological control of velvetleaf.

Bugs of all stages, feeding on seeds, were present in cages. Seeds attacked by *Liorhyssus hyalinus* were lighter in color, smaller in size and had sunken areas compared to non-attacked seed. Microscopic examination of seed from infested plants revealed depressions in or puncture holes through seed coats. On seed and capsules, reddish to orange-red feces were observed. Damaged seed would show reductions in seed viability because when it was cut, seed content was sucked dry.

In laboratory experiment, in which bugs were isolated directly on mature seed in covered pots, no feeding was recorded, and the bugs did not survive. This could explain why the greatest number of bugs in field observation preferred immature (green) capsules.

Homoptera

The aphid species *Rhopalosiphum padi* was collected in August 2000 in the locality Lupoglav, *Macrosiphum euphorbiae* in August 2000 in Zupanja and *Aphis fabae* in July 1999 in Zagreb. Adults were collected from leaves and stems, but no damage on plants was recorded during field observations. As several specimens of mentioned species were present on velvetleaf plants, it is possible that velvetleaf was their food plant. However, the aphids died during transportation to the greenhouse and feeding on velvetleaf could not be confirmed.

Coleoptera

In field observation of velvetleaf plants 2 Coleoptera species were recorded: *Psylliodes brisouti* and *Podagrica menetriesi*. However, only one specimen of *P. brisouti* was found on velvetleaf in 1997, and no damage was recorded. This

species is considered to be an accidental on velvetleaf plants. The finding of *Psylliodes brisouti* was the first record of this species in Croatia.

Podagrica menetriesi Falderman, 1837

This species was found in the localities Stari Mikanovci and Otok, feeding on leaves of velvetleaf. According to literature data, this species was recorded on plants from the Family Malvaceae Juss. (*Althaea officinalis* L, *A. rosea* (L.) Cav, *A. apterocarpa* Cihac, *A. pallida* Wald. & Kit, *Malva sylvestris* L, 1753, *M. neglecta* Wallr, 1824, *M. arborea* A. St.-Hil, 1827) (Nonveiller, 1960; Medvedev & Roginskaya, 1988). This is the first record of *P. menetriesi* on velvetleaf.

In field observations, adults of *Podagrica menetriesi* feeding on leaves of velvetleaf were recorded on September 13th 1996 and at the end of August (August 22nd) 1997 in the locality Otok, as well as in September (September 3rd) 1997 in the locality Stari Mikanovci. Because this species was found with a great number of specimens on velvetleaf plants, insects were collected and transported to the greenhouse. Feeding on velvetleaf leaves, as well as on the leaves of the weed species *Malva neglecta* and the field crops *Zea mays* L., *Brassica napus* L. subsp. *oleifera* DC Metzger and *Beta vulgaris var. saccarifera* ALEF, in which velvetleaf is present as a weed, was tested in the greenhouse. The results of the feeding test are presented in Table 3. Observation of insects in the greenhouse was performed in the period August 22nd - November 6th 1997. Decline in food consumption was recorded at the end of September and at the beginning of October 1997, after what diapauses of species in greenhouse conditions started.

Adults of *Podagrica menetriesi* were present in groups up to 10 specimens/ leaf. While feeding on leaves, insects caused oval-shaped holes up to 4-6 mm^2. One imago made about 5 holes/day, so in a presence of a few specimens, significant leaf damage was recorded.

Lepidoptera

In this investigation a total of 5 Lepidoptera species were collected. In literature data species *Crocidosema plebejana*, *Heliothis armigera* and *Ostrinia nubillalis* were recorded as insects associated with velvetleaf, while *Carcharodus alceae* and *Autographa gamma* were recorded on this weed for the first time. During field observations, all species were collected in caterpillar stage. Caterpillars

were collected and taken to greenhouse observation and feeding testing. Species *Ostrinia nubillalis* and *Autographa gamma* are well known pests on field crops, and because of their occasional appearance on velvetleaf, in infested corn and sugar beet where velvetleaf was present as a weed, those species were considered as an accidental species.

Caterpillars of *Autographa gamma* were collected from velvetleaf in the locality Cepin, at the end of June (June 25th) 1996, in sugar beet where damages caused by this polyphagous pest were recorded. In greenhouse, caterpillars were feeding on velvetleaf leaves and in a few days (June 28th) species pupated. In 13 days (July 11th) butterflies were developed and identification of the species was confirmed.

Caterpillars of *Ostrinia nubillalis* were collected in the localities Stari Mikanovci, Vrpolje and Lupoglav from velvetleaf plants in corn field in 1998. Caterpillars were feeding in stems and branches of velvetleaf. Up to 20 cm long holes were measured in stems. Because this pest was recorded on mature velvetleaf plants that had already produced seed, and competition from velvetleaf in the formation of the corn yield had finished for that year, the role of *Ostrinia nubillalis*, even as an accidental biological control agent in velvetleaf control, was almost negligible.

Carcharodus alceae Esper, 1780

Carcharodus alceae (Mallow Skipper) is widely distributed species, from North Africa and Spain through South and Central Europe to Asia. It is absent from Denmark, Fennoscandia, the Baltic countries and Britain (Higgins & Riley, 1970). In Belgium it is an extinct species, while in Switzerland and Germany it is threatened species.

According to faunistic data for Croatia, it is widely distributed in continental, central-mountain, Mediterranean regions and on many islands (Mladinov, 1965; Mladinov, 1967; Mladinov, 1971; Bartol et al., 1964; Burgermaister, 1964; Kranjčev, 1985).

In this investigation *C. alceae* was recorded in total of 24 localities from 1995 to 2000. In a total of 22 localities in Croatia (Magadenovac, Cepin, Grabovac, Knezevo, Nustar, Vinkovci, Stari Mikanovci, Vrpolje, Otok, Komletinci, Bosnjaci, Posavski Podgajci, Drenovci, Tovarnik, Kutjevo, Nova Gradiska, Gradina, Dragicevac, Popovaca, Hercegovac, Grabrov Potok, Lupoglav, Ivanic Grad and Zagreb) this species was recorded for the first time.

As food plants of *C. alceae*, in literature data, plants from the genus *Malva* L, *Althaea* L, *Hibiscus* L. were recorded (Higgins & Riley, 1970). In this investigation velvetleaf was newly recorded as a food plant for *C. alceae* from the genus *Abutilon* P. Mill.

In field observations, eggs of this species were collected with leaves of velvetleaf and were transported to the greenhouse. In the greenhouse, caterpillars of this species were developed, and continued to feed on fresh leaves of velvetleaf.

Frequently, caterpillars of *C. alceae* were found on velvetleaf leaves, and were collected for further investigation in the greenhouse.

In field observations, caterpillars of *C. alceae* on velvetleaf plants were recorded from April to November, with greater abundance from the second decade of July to the second decade of September.

In the laboratory, the observed caterpillars of *C. alceae* pupated, and the butterflies that were developed successfully were identified. Butterflies usually died within 6 days of emerging from pupa. No copulation or oviposition was recorded in the laboratory.

Some caterpillars hibernated in the greenhouse on leaves of velvetleaf, and their activity was recorded again in the first decade of February. With no further feeding, caterpillars pupated and the first appearance of butterflies was recorded in the first decade of March. If the appearance of butterflies in field conditions is about the mentioned time, the first occurrences of caterpillars on velvetleaf recorded in Croatia in the beginning of April, could be a logical succession.

Caterpillars of *C. alceae* feed in specific overlaps on leaves, which they make by their own activity. As the result of caterpillar feeding, the leaf area was damaged. In field conditions, two or three caterpillars/leaf were often recorded. Isolated on a leaf area (about 65 cm^2), two caterpillars damaged about 20% of the leaf area in 3 days. It was also recorded that caterpillars abandon a leaf which they do not eat in its totality. Such leaves start to wither.

The feeding of *Carcharodus alceae* caterpillars was tested on velvetleaf and on 9 other plants of the Family Malvaceae and some field crops plants of the Families Chenopodiaceae Vent, Brassicaceae Burnett, Fabaceae Lindl. and Poaceae Barnhart (Table 4).

Crocidosema plebejana Zeller, 1847

The species *Crocidosema plebejana* is a widely distributed and common species in Southern Europe, Africa, throughout the Pacific islands to Australia and New Zealand, Japan, North and South America (Bradley et al. 1979).

According to the insect fauna collection of the Croatian Natural History Museum, this species was recorded and collected in one Croatian locality, Garcin, in 1902.

In this investigation, the species *Crocidosema plebejana* was recorded in 6 new localities: Stari Mikanovci, Otok, Vrpolje, Lupoglav, Grabov Potok and Popovaca in 1997 and 1998.

In field observations of velvetleaf plants, caterpillars of *C. plebejana* were recorded in stems, flower buds and seed capsules, from July to the first decade of October, when pupas were recorded.

In greenhouse, formation of pupas was recorded from the beginning of September to the second decade of October. Pupas were recorded in seed capsules and also in dry leaves, in the field and in the greenhouse.

Adults of this species in the field were recorded in the first decade of September.

In greenhouse, the first adults, emerged from pupas were recorded in September (September 16th) 1997, October (October 10th,17th,21st,24th and 29th) 1997 and November (November 4th, 14th and 15th) 1997.

In September and October, butterflies died within 4-6 days of emerging from the pupa. No copulation and ovipositioning were recorded in the cages on the fresh plants growing in pots.

The presence and feeding of *Crocidosema plebejana* on stems and branches of velvetleaf were detected from traces of caterpillar faeces near perforations on the mentioned plant parts. On one stem up to 7 perforations were recorded, while at the same time, borings in flower buds and capsules could be observed.

In the field, the wilting of smaller, damaged plants (up to 60 cm) and leaves falling from bigger plants were recorded.

Caterpillar feeding on flower buds and seed capsules causes a reduction in seed production. One larva can eat the content of one flower bud /day. It was also measured that the content (soft white seeds) of green (immature) seed capsules would be damaged in two days. If the seed capsules were harder (not completely mature) and the seeds were mature and harder the caterpillar would damage only a few seeds in the seed capsule, while more seeds were undamaged.

Heliothis armigera Hübner, 1808

This species is a general feeder pest worldwide in cotton, tobacco, maize, tomato, potato, pepper, sunflower, sorghum, soybean, beans, chick pea, globe artichoke, lettuce, cucurbit, alfalfa, leek, plum, citrus, as well as on ornamental plants and flowers, including pinks and geraniums.

In this investigation *H. armigera* was established as a feeder on velvetleaf plants in the localities Otok, Lupoglav, Drenovci and Tovarnik in 1996, 1997 and 2000. According to the literature, this species is widespread in Croatia.

Caterpillars were recorded on velvetleaf seed capsules from the third decade of August (caterpillars up to 8 mm long) to the third decade of October (caterpillars up to 25-30 mm long) in different development stages.

The main damage was due to caterpillar feeding on green seed capsules, but feeding on leaves was also recorded. In greenhouse observations, it was recorded that one caterpillar (25-30 mm long) could eat 18-24 seeds from 6-8 capsule partition/seed capsule. As the average mass of 1 immature seed is about 0.02 g, one caterpillar could eat about 0.36-0.48 g of seed/day. Smaller flower buds were eaten completely in one day.

Feeding on leaves of velvetleaf was rarely recorded, but in greenhouse observations it was measured that one caterpillar (15 mm long) could eat about 50% of one leaf area in five days. Also, 3 caterpillars (15 mm long) eat 75% of one leaf in 3 days.

Discussion

In the investigation of insects as potential biological control agents for velvetleaf control conducted in Croatia from 1995 to 2000 a total of 24 Croatian localities in 9 Croatian counties were surveyed. Similar investigations were conducted in the USA (Gibb, 1991; Headrik et al., 1997 cit. Lesnik, 1999; Kingslover et al., 1989; Mitich, 1991; Zheng et al., 2004), Italy (Pemberton & Hoover, 1980), Greece (Pemberton & Hoover, 1980) and Slovenia (Lesnik, 1999). According to literature data (Table 1) a total of 32 insect species were recorded associated with velvetleaf. Unfortunately, none of these species could be used as biological control agents for velvetleaf control by classical or any other biological control approach.

A total of 15 insect species were recorded as being associated with velvetleaf in Croatia (Table 2). Among the 15 species mentioned, a total of 10 insect species

(*Carpocoris purpureipennis*, *Graphosoma lineatum italicum*, *Lygeaeus saxatilis*, *Pyrrhocoris apterus*, *Rhopalosiphum padi*, *Macrosiphum euphorbiae*, *Podagrica menetriesi*, *Psylliodes brisouti*, *Carcharodus alceae* and *Autographa gamma*) have been recorded on velvetleaf for the first time. For all collected species, the new localities of distribution in Croatia were recorded. For the species *Podagrica menetriesi*, *Carcharodus alceae* and *Autographa gamma*, velvetleaf was confirmed to be a new food plant. For the other 7 of a total of 10 new species recorded on velvetleaf in Croatia, feeding was not confirmed in the greenhouse.

The finding of species *Psylliodes brisouti*, which was recorded on velvetleaf as an accidental species, was the first record of this species in Croatia.

From 15 insect species collected on velvetleaf in Croatia only 5 frequently collected species (*Liorhyssus hyalinus*, *Podagrica menetriesi*, *Carcharodus alceae*, *Crocidosema plebejana* and *Heliothis armigera*) were investigated in more detail.

The species *Liorhyssus hyalinus* was recorded in the localities Lupoglav, Otok, Vinkovci and Stari Mikanovci mostly on green immature seed capsules of velvetleaf. The symptoms on seed caused by the feeding of this species were identical to those recorded in the USA, where seed damaged by the feeding of this species did not germinate (Gibb, 1991). In Croatia, the seed content of velvetleaf seed was sucked and there was an undoubted reduction in seed viability of all seeds in the seed capsule. As all development stages of *L. hyallinus* were recorded in the field and also in the greenhouse on transplanted plants of velvetleaf, this weed species was found to be "host" and "food plant" of *Liorhyssus hyalinus*. Because this species is easy to rear in the greenhouse and is a highly efficacious seed-feeding species, its use as potential biological agent for velvetleaf control by the augmentative approach (Frick & Chandler, 1978) should be discussed. It should be possible as long as no conflict of interest arises in certain situations, since it is known from literature that *Liorhyssus hyalinus* can feed on plants from the genera *Mesembryanthemum*, *Sonchus*, *Helianthus* and *Zea*.

The species *Podagrica menetriesi* was found in the localities Otok and Stari Mikanovci and it is newly recorded insect species associated with velvetleaf. In feeding tests conducted in the greenhouse, feeding of *P. menetriesi* on leaves of velvetleaf and of the species *Malva neglecta* and *Brassica napus* subsp. *oleifera* was recorded (Table 3). In spite of leaf damage recorded in field observation,

seed production was not affected, but the competitive ability of velvetleaf in field crops could be decreased.

The species *Carcharodus alceae* is also a newly recorded insect species associated with velvetleaf. It was the most frequently collected species on velvetleaf plants. In 5 years of investigation it was recorded in a total of 24 Croatian localities (Table 2). The investigation of the biology of *Carcharodus alceae*, combined through greenhouse and field observation, indicated the complete life cycle of this species on the velvetleaf, although the velvetleaf was not found only as a food plant but also as a host plant of *Carcharodus alceae*.

Because of its detected feeding on some ornamental Malvaceae plants in this investigation (Table 4) as well as on some medicinal herbs and ornamental plants mentioned in the literature (Higgins & Riley, 1970), this phytophagous species could not be recommended as biological control agent capable of being used in classical biological control of the velvetleaf. Although no decrease of seed production/plant was recorded, the impact of *Carcharodus alceae* on the weakening of the competitive ability of velvetleaf in field crops through its feeding on the leaves of this weed cannot be neglected. Because this species is widely distributed in Croatia and frequently present on the velvetleaf as host and food plant, the use of *Carcharodus alceae* in velvetleaf control by the augmentative approach should be considered, at least where no conflict of interests is likely to appear.

The species *Crocidosema plebejana* was recorded in 6 localities: Stari Mikanovci, Otok, Vrpolje, Lupoglav, Grabov Potok and Popovaca. It is an effective seed predator, but also has a great impact on the survival of plants in

Table 3. Testing of feeding of species *Podagrica menetriesi* on leaves of different plant species in the greenhouse

Family	Species	Feeding test
Chenopodiaceae	*Beta vulgaris* L. var. *saccharifera* ALEF.	-[a]
Brassicaceae	*Brassica napus* L. subsp. *oleifera* DC Metzger	+[b]
Malvaceae	*Abutilon theophrasti* Medik.	+
	Malva neglecta Wallr.	+
Poaceae	*Zea mays* L.	-

[a] Negative feeding test, insects did not feed on plant species leaves
[b] Positive feeding test, insects fed on plant species leaves

Table 4. Feeding test of the species *Carcharodus alceae* on leaves of different plant species in the greenhouse

Family	Species	Feeding test
Chenopodiaceae	*Beta vulgaris* var. *saccharifera* ALEF.	-[a]
Brassicaceae	*Brassica napus* L. subsp. *oleifera* DC Metzger	-
Malvaceae	*Althaea officinalis* L.	+[b]
	A. cannabina L.	-
	A. rosea Cav.	+
	Hibiscus syriacus L.	-
	H. manihot L.	-
	H. palustris L.	-
	H. syriacus L.	+
	Malva neglecta Wallr.	+
	Kitaibela vitifolia Willd.	+
	Abutilon theophrasti Medik.	+
Fabaceae	*Glycine max* L.	-
Poaceae	*Zea mays* L.	-

[a] Negative feeding test, caterpillars did not feed on plant species leaves
[b] Positive feeding test, caterpillars fed on plant species leaves

the earlier phase of velvetleaf development. As this species could feed on some other Malvaceae plants from genus *Althaea*, *Hibiscus*, *Gossypium* L, *Malva* and *Crataegus* L., its use as classical biological control agent could not be recommended. But the contribution of the species *Crocidosema plabejana* in velvetleaf control in field conditions is not negligible.

The impact of the species *Heliothis armigera* on velvetleaf was observed in more detail in greenhouse conditions, but because this species is a well-known general feeder pest worldwide, its use as potential biological control agent for velvetleaf control cannot be discussed. However, its role in the reduction of number of flower buds and number of seed in green seed capsules in field conditions is of great significance.

In this investigation no insect was found capable of being a classical biological control agent for velvetleaf. The future use of species *Liorhyssus hyalinus*, a highly efficacious seed-feeding species as well as of *Carcharodus alceae* as an effective leaf-feeding species in velvetleaf control by augmentative approach should be considered. However, the feeding of several insect species at the same time was frequently recorded: *Carcharodus alceae* (leaves), *Crocidosema plebejana* (stems, branches, flower buds, seed in seed capsules), *Heliothis armigera* (leaves,

green seed capsules) and *Liorhyssus hyalinus* (seed in seed capsules) on different velvetleaf plant parts. Insect fauna found feeding on velvetleaf decrease the competitive ability of this weed in field crops and/or reduce the number of viable seed/plant, which is a valuable help in the chemical control of this weed. In this way, biological control agents (in our investigation, insects) must be seen as stress-factors, not as weed killers and biological weed control must be regarded as an integral part of a well-designed pest management strategy, not as a cure by itself (Müler-Schärer, 1995; Müler-Schärer & Frantzen, 1996).

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