



Milkweed control by food imprinted rabbits



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ABSTRACT

Many species of invasive plants are spreading out rapidly in Europe. The common milkweed occupies increasingly more area. Being poisonous, most animals will not graze on it however rabbits would be an effective organism for the biological control of milkweed. Rabbit kittens can learn the maternal diet in various ways. They prefer aromatic foods which their mother had eaten during pregnancy or lactation period, -even if it is poisonous- but they can also learn the maternal diet from the fecal pellets deposited by the mother into the nest during the nursing events. The present study was aimed to investigate if rabbit kittens can learn that the common milkweed is a potential food also. In the first 10 days of their lives kits got fecal pellets originating from individuals having fed on common milkweed previously. When weaned on day 28 postpartum, these pups preferred the milkweed in the 3-way food choice test, opposite to the control group. Most surprisingly in a second experiment it was also shown that the common milkweed was also preferred by the kittens if their mother ate it not during, but one month before pregnancy.

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1. Introduction

The invasion of exotic plant species has become very important in Europe especially in Hungary, because Hungary is situated in a transitional biogeographic zone between the deciduous forest and the forest steppe in the Carpathian Basin, where several species reach the borders of their distribution. Furthermore, Hungary is a gateway for invasion as well. Numerous alien plant species have been found in Hungary, for instance *Ailanthus altissima*, *Asclepias syriaca*, *Ambrosia artemisiifolia*, *Aster* spp, *Celtis occidentalis*, *Vitis riparia* (Török et al., 2003) among many others.

The spread of aggressive invasive plant species is becoming crucial both from a plant defense and nature protection point of view. The majority of studies found larger values for population size, plant density, vigor, reproductive output, and seed bank size in the exotic place compared to the native range (Hinz and Schwarzaender, 2004). These plant species spread quite rapidly and aggressively and can hardly be controlled (Lamming, 2001), because they can be released from biotic constraints like natural enemies (Keane and Crawley, 2002; Shea and Chesson, 2002) and competitively superior neighbors, such as plant competitors adapted to allelopathic effects (Bais et al., 2003; Callaway and Aschehoug, 2000). Invasive plants are harmful not only for fields but also for nature reserves

(Lodge, 1993). There are basically three methods for controlling invasive species, 1. use of chemicals, 2. cutting or 3. grazing.

Milkweed (*Asclepias syriaca* L) is an invasive species originating from North America. The aggressive spread of this species endangers the local endemic vegetation (Szöke, 2001). This plant can propagate easily due to the lack of the otherwise few enemies known in its natural habitat (10 herbivore insect species were described on their natural habitat, Malcolm, 1991; Agrawal and Malcolm, 2002). Common milkweed has a milky white latex that is a mechanical defense against herbivores (Dussourd and Eisner, 1987; Malcolm, 1991) and this plant species contains cardenolides, too (Malcolm, 1991), that makes milkweeds distasteful or toxic to mammalian herbivores and some generalist insect herbivores as well (Malcolm, 1991). Furthermore Malcolm and Zalucki (1996) also demonstrated significantly positive cardenolide induction one day after manually imposed partial leaf defoliation on milkweed. So the common milkweed is a poisonous species, it is difficult to have it grazed or eaten up spontaneously by most of the mammals and insect herbivores. One method for the control of invasion is removing the plants twice in a year either by plugging it out or cutting and collecting the green material to prevent the germination of seeds. This is important, as it is known that the seeds keep the ability to germinate and grow into a healthy plant even after a period of seven years (Csontos et al., 2009). However, cutting requires a lot of effort both of energy and of time, it is expensive, and still doesn't provide a satisfactory long term effect. Another possibility for milkweed control is using chemicals (ie. Medallon is a substance selective to dicotyledonous species). But still, these substances usually affect

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other dicotyledonous species as well, and the effect of chemicals is not long lasting (Sztár and Török, 2008). In the Kiskunság National Park, milkweed had been found in one single patch with a 10 m diameter in 1994 (Kertész et al., 1993), then in 2011, it was realized that the original minor patch increased approximately 100 m in diameter, and numerous additional patches of milkweed had emerged. On other parts of the Kiskunság National Park, chemical control of milkweed is in progress as a part of a LIFE Project. It has become clear by now that chemical control is time-consuming and expensive. Furthermore, it has had some unexpected negative effect on other parts of the vegetation (Sztár and Török, 2008).

Although, in the case of several other invasive species grazing as a biological method should be taken into account as in fact, it has been used as a method for defense for centuries (Csecserits and Rédei, 2001). Invasive species can effectively be suppressed by grazing on a given habitat. In the case of persistent grazing, the invasive species is drained and dies (Lamming, 2001). This is because weeds primarily invade loose soil (Bagi et al., 2007) but avoid physically undisturbed open grasslands. Being a seriously poisonous species, it is difficult to have *Asclepias* grazed or eaten up spontaneously, therefore the third method-grazing is excluded. Or is it?

Our present results might provide a simple biological solution: our earlier investigations conducted both in the field and in the laboratory revealed that rabbit grazing can substantially alter the vegetation composition, (Kertész et al., 1993) It was found, that rabbits show a different dietary tradition in different habitats possibly as a consequence of the social transmission of food preferences (Mátrai et al., 1998; Hudson et al., 1999). Micro- histological analyses of the fecal pellets collected from the different herbivore species throughout the year, revealed basic differences, between two regions of the Kiskunság National Park, Hungary. While a major part of the winter food of rabbits was juniper in the Bugac region that was not the case in Bócsa (Mátrai et al., 1998). Surprising results, considering, that juniper is poisonous to some extent. To check whether differences in the grazing habit of the rabbits were caused by the different substance composition of juniper plants originating from the two regions, young juniper plants from the Bócsa region were placed in the Bugac Forest in a pot. Half of them were fenced while the rest were left free. It turned out during several weeks, that all of the unfenced plants originating from Bócsa disappeared by grazing in Bugac, while the fenced individuals remained untouched. The difference in the juniper grazing habit is a consequence of the different feeding traditions of the rabbits and not the difference in juniper shrubs inhabiting the two regions. (Markó et al., 2008)

Our hypothesis was supported by results of a series of laboratory experiments. When the diet of laboratory mothers was supplemented either with juniper berries or thyme leaves, both of which are eaten by rabbits in the Bugac region (Mátrai et al., 1998), kittens later preferred the aromatic plant that their mother had eaten. Measuring the pups' food consumption in a three-way simultaneous food-choice test following weaning for one week, treated pups preferentially chose the aromatic food in question and digested significantly larger amounts compared to the control litters. Relative juniper consumption of juniper treated pups was not only significantly larger than those of control litters, but exceeded the 10% maternal intake (Altbäcker et al., 1995). These results clearly indicate that, despite the lack of extensive maternal care in rabbits, information about certain foodstuffs can be transmitted from mother to young already in the nest, prior to weaning. It was also supported that although maternal care in rabbits is limited to the 3 min nursing per day, there are at least 3 sources of the information about the mother's diet. 1. the amniotic fluid, which might contain several substances from the mother's diet during pregnancy 2. the mothers milk, because certain aromatic substances can pass to the milk during the lactation period, or 3. the fecal pellets deposited

to the nest by the mother during the nursing events (Bilkó et al., 1994). These pellets also contain some remnants of the plant previously eaten by the mother, and as kits not only sniff, but also eat these maternal pellets, they might serve as information (Bilkó et al., 1994). The finding that offspring show a preference to foods having been eaten by their mother even if it is toxic (Altbäcker et al., 1995) and the effect is long lasting suggests that such early learning has strong, imprinting-like characteristics (Hudson and Altbäcker, 1992). If this is the case, offspring are able to remember and show a preference for their mother's diet not only some weeks, but even some months following weaning, even if they had no direct experience with these solid foodstuffs meanwhile. And in fact results of further experiments revealed also that this is a long lasting effect influencing the offspring food choice even weeks or six months later, even if they have no direct access to the food in question in the meantime (Bilkó, 1994).

Given these results, the question emerged, whether the social transmission of food preferences, if directed cleverly, could provide an excellent possibility to control the spread of several invasive plant species?

Therefore in the current study we searched for the answer to 3 basic questions:

2. Are rabbits willing to eat *Asclepias syriaca* spontaneously?

2.1. Can milkweed preference be induced by social learning in the lab?

Relying on our earlier findings we conducted an experiment to answer whether the rabbits would eat milkweed spontaneously and if so, whether pellets originating from a milkweed fed individual can affect the food choice of the offspring?

3. Can young animals learn a milkweed preference if the mother ate it not during the pregnancy and lactation period, but one month before the onset of gestation?

2.2. Experiment 1

In this experiment we aimed to answer the first two questions: Do rabbits eat milkweed spontaneously in the lab, and if so can we induce the habit of milkweed (*Asclepias syriaca* L) consumption in young rabbits by replacing mother's fecal pellets with fecal pellets originating from an *Asclepias* fed individual? This method utilized the fact that rabbit does defecate into their nests during nursing and their kits readily sniff and eat these pellets (Hudson et al., 1996). This method was chosen, because it also prevented the possible accumulation of milkweed remnants in the mothers' body (Bilkó et al., 1994), and ensured that possible adverse effects did not reach the offspring.

3. Materials and methods

Experimental subjects were pregnant wild rabbits and their offspring. The mothers were kept individually in standard wire rabbit cages with water and lab food (Altromin, LATI Ltd, Gödöllő Hungary) freely available. During the experiment, a 14/10- h light/dark cycle was maintained at 19–22 °C. The rabbit mothers and their litters were randomly allocated into 2 different treatment groups, labelled as *Asclepias* group and Control group. We expected that the exposure to fecal pellets containing remnants of different food stuffs affects pups later food choice and the pups' preference will be reflected in the relative amount of each food eaten.

Parturition and nursing procedure: As the time of parturition was approaching, a plastic nest box (30 × 45 × 25 cm) was fitted

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