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Parasitoid inventarisation of European corn borer (*Ostrinia nubilalis* Hübner, 1796) and options for its biological control in Slovenia

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ABSTRACT

European corn borer (*Ostrinia nubilalis*) (ECB) is an important maize pest in central and northern Europe. Presently it is controlled by insecticides or biological agents such as *Trichogramma brassicae* in several European countries, excluding Slovenia, where the pest's pressure is highly variable and no appropriate mechanization is available. Lessening the dependence on chemical pesticides is an integral part of the European Union's agenda for agriculture. Mass release of *Trichogramma* spp. could be seen as a promising alternative for ECB control in countries with a highly fluctuating ECB pressure and no mechanization for insecticide applications. However, no records of naturally occurring hymenopteran parasitoids of ECB exist in Slovenia. To address this important under-researched topic and provide the expert basis for potential introduction of ECB egg parasitoids in Slovene maize production, a systematic inventarisation programme of ECB parasitoids was launched in 2010. Additionally, ECB flight was monitored in 2011 and 2012 at two locations in Slovenia: Jablje and Rakičan. In both locations two ECB generations were observed. ECB was fist observed at the end of May in Rakičan. During the five years of the systematic survey we discovered two ECB parasitoid species. ECB egg masses were parasitized by *Trichogramma brassicae*, whereas ECB pupae were parasitized by *Tycherus nigridens*, with 6 or 7 % parasitation rate, respectively. *T. nigridens* represents a new taxon report for Slovenia. We conclude that there is a strong need for undertaking systematic surveys of natural enemies of agricultural pests.

Key words: biological control; corn; Ichneumonidae; insect pests; maize; new records; parasitoids; *Tycherus nigridens*; Trichogrammatidae; *Trichogramma brassicae*; *Zea mays*

IZVLEČEK

INVENTARIZACIJA PARAZITOIDOV KORUZNE VEŠČE (*Ostrinia nubilalis* Hübner, 1796) IN MOŽNOSTI NJENEGA BIOTIČNEGA ZATIRANJA V SLOVENIJI

Koruzna vešča (*Ostrinia nubilalis*) (ECB) je pomemben škodljivec koruze v srednji in severni Evropi. V številnih evropskih državah jo obvladujejo z insekticidi, kar pa ne velja za Slovenijo, saj poleg tega, da je populacijski pritisk tega škodljivca zelo spremenljiv oziroma nepredvidljiv, tudi ni na voljo ustrezne škropilne tehnike. Glede na zastavljene cilje EU, ki so usmerjeni v zmanjševanje tveganja zaradi rabe fitofarmacevtskih sredstev, je uporaba biotičnih agensov, kot je na primer množični izpust parazitoidov iz rodu *Trichogramma* proti ECB dobrodošla in obetavna alternativa kemičnim sredstvom. Znanje o zastopanosti in razširjenosti parazitoidov ECB iz reda kožekrilcev je v Sloveniji še vedno pomanjkljivo. Za preučitev možnosti obvladovanja ECB z biotičnimi agensi smo leta 2010 začeli sistematično spremljati navzočnost in razširjenost parazitoidov ECB na koruznih poljih. Dodatno smo v letih 2011 in 2012 spremljali nalet ECB v Jabljah in Rakičanu. Na obeh lokacijah smo odkrili dva rodova ECB. Škodljivec se je navadno pojavil najprej v Rakičanu konec maja. V petih letih raziskave smo v Sloveniji naleteli na dve vrsti parazitoidov ECB, in sicer na vrsto *Trichogramma brassicae*, ki smo jo izolirali iz parazitiranih jajčec ECB in na vrsto *Tycherus nigridens*, ki smo jo izolirali iz parazitiranih bub ECB. Stopnja parazitizma je bila 6 % za *T. brassicae* in 7 % za *T. nigridens*. Vrsta *T. nigridens* predstavlja novo taksonomsko najdbo za Slovenijo. Zaključujemo, da je izvajanje sistematičnega iskanja in inventarizacije naravnih sovražnikov kmetijskih škodljivcev nujnega pomena za Slovenijo.

Ključne besede: biotično varstvo; Ichneumonidae; koruza; nove najdbe; parazitoidi; *Tycherus nigridens*; škodljivci koruze; Trichogrammatidae; *Trichogramma brassicae*; *Zea mays*

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1 INTRODUCTION

Corn borers represent an important biotic stressor for maize (*Zea mays* L.) crops in Europe (Meissle *et al*., 2010). European corn borer (*Ostrinia nubilalis* (Hübner 1796)) (ECB) is the most important maize pest in central and northern Europe, while pink stem borer (*Sesamia nonagrioides* (Lef., 1827) is predominant in warmer areas of southern Europe (Velasco *et al*., 2002; Malvar *et al*., 2004; Meissle *et al*., 2010). ECB mostly causes damage by larvae that enter into the maize stalk after hatching and feed on the stalk pith. Yield is affected by ECB tunnelling which interferes with vascular system and increases the risk of stalk lodging and breakage (Gomboc *et al*., 1999). In addition, corn borer damage can affect plant health by vectoring *Fusarium moniliforme* J. Sheld. and facilitating fungal infections (Sobek and Munkvold, 1999). The yield loss produced by corn borer attack of the ear is sometimes less important than yield reduction associated to stalk tunnelling, as this kind of damage has been described as an important factor for favouring high levels of fumonisins in maize kernels (Sobek and Munkvold, 1999; Butrón *et al*., 2009).

Foliar insecticide applications in maize production are used in most European regions (i.e. Spain, Hungary, Poland, Germany, Italy, France and Denmark). The most commonly used active ingredients are pyrethroids and organophosphates (Meissle *et al*., 2010). However, Pons and Albajes's (2002) results illustrate how broad spectrum insecticides can have undesirable effects regarding ECB control: although treating maize seeds with imidacloprid reduced the incidence of cutworms (*Agrotis segetum* Denis & Schiffermüller, 1775), wireworms (*Agriotes lineatus* Linnaeus, 1767), pink stem borer and leafhoppers (*Zyginidia scutellaris* Herrich-Schäffer, 1838), treated plots were attacked significantly more by ECB. In Slovenia however, chemical measures in corn crops are not undertaken against ECB, partly due to highly fluctuating ECB pressure, partly due to non-existent spraying mechanization. It is important to mention that ECB in Slovenia occurs also in vegetables like tomato, peppers and others, where the pest can be controlled with insecticides (Carlevaris *et al.,* 2003).

Lessening the dependence on chemical pesticides is an integral part of the European Union's (EU) agenda for agriculture (European Parliament, 2009). The objective of this Directive is to reduce dependence on, as well as the risks and adverse impacts of, pesticide use on human health and the environment, and a key element to reach this goal is to promote the implementation of Integrated Pest Management (IPM), which has become compulsory in the EU in 2014. In sync with this directive and the previously mentioned reasons mass release of *Trichogramma* spp. is a promising alternative for ECB control in countries with a highly fluctuating ECB pressure and no insecticide applications. In Europe, the parasitoid wasps are already released mainly against ECB on about 150 000 ha per year with the largest area in France (Meissle *et al*., 2010). Encouraging results of biological control of ECB also exist from Asia (Zhang *et al*., 2010) and North America (Hoffmann *et al*., 2002; Hoffmann *et al*., 2006). An important ECB parasitoid, *Trichogramma brassicae* Bezdenko, 1968, was only recently discovered in Slovenia by Bohinc *et al.,* (2015).

Despite encouraging results of biological control of ECB, records of naturally occurring hymenopteran parasitoids of ECB in Slovenia are limited. To address this important under-researched topic and provide the expert basis for potential introduction of ECB egg parasitoids in Slovene maize production, a systematic inventarisation programme of ECB parasitoids was launched in 2010. In this paper we report the first findings.

2 MATERIALS AND METHODS

2.1 Locations

From 2010 to 2014 a systematic search for natural enemies of ECB was carried out at different locations in central Slovenia (Jablje near Ljubljana) and the north-eastern part of Slovenia (Rakičan, Prekmurje) with predominant maize production. Several fields within Agricultural Institute's Jablje experimental station were monitored (46°08'17.1"N, 14°34'15.2"E; 46°08'25.3"N, 14°34'39.8"E; 46°09'05.8"N, 14°34'52.5"E and 46°08'20.6"N, 14°34'15.0"E) and two locations near the Biotechnical School Rakičan (46°38'53.2"N, 16°13'21.3"E and 46°39'17.1"N, 16°11'33.1"E). The exact parasitoid finding dates are listed in Table 1.

Table 1: Dates and locations of parasitoid discoveries in the years 2010-2014

	Jablje			Rakičan		
Year	*T. brassicae*	*T. nigridens*	Maize plants surveyed	*T. brassicae*	*T. nigridens*	Maize plants surveyed
2010	/	/	200	/	/	/
2011	Jul. 8	Aug. 3	770	Jul. 7 and Aug. 1	/	760
2012	Aug. 7. and Aug. 21	/	520	/	Avg. 21	320
2013	/	/	400	/	/	/
2014	/	/	400	/	/	/

2.2 ECB flight monitoring

In the sampled areas (Jablje, Rakičan) ECB flight was monitored with a fluorescent light trap (Grote Lichtval zonder lamp, Entomologie-speciaalzaak, Vermandel, Hulst, Nederlands) from May to September in the years 2011-2012. One trap per location was used.

2.3 Scouting for ECB egg masses and pupae in the field

Our search for natural enemies of ECB was focused on egg and pupal parasitoids. Search for ECB egg parasitoids was performed by scouting for ECB egg masses on the abaxial side of maize leaves. Scoutings were carried out during the oviposition period of the second ECB flight. All potentially parasitized ECB egg masses were collected and transferred to the entomological laboratory of Agricultural Institute of Slovenia for observation of parasitoid emergence. Pupal parasitoids were searched for in plants with evident ECB damage (stalk tunnelling and breakage, frass on leaves, holes in leaves). ECB damaged plants were dissected to collect ECB pupae, which were also transferred to the entomological laboratory of Agricultural Institute of Slovenia for observation of pupal parasitoid emergence.

2.4 Observation of ECB egg masses and pupae for parasitoid emergence

ECB egg masses on 10 cm^2 pieces of maize leaves and ECB pupae were put into individual 50 mL centrifuge tubes. The tubes were kept at room temperature for observation until ECB larva/imago or parasitoid emerged.

2.5 Parasitoid classification

The morphological classification of the ECB egg mass parasitoids was based on Cônsoli *et al*. (2010), Ferriere and Kerrich (1958), Goulet and Huber (1993) to family and genus level. For species identification, Pintureau (2011) was used. Identification of *Trichogramma* species relies very heavily on examination of the male genitalia and to a lesser extent the male antennae. For this reason a slide-mounting technique modified from Platner *et al*. (1999) was necessary to allow the examination of male genitalia. Therefore, freshly emerged individuals of the egg parasitoid were killed and incubated in 90 % lactic acid for three days in order to allow their clearing. Then specimens were transferred, dorsum-up, to a small drop of Hoyer's medium on a slide and carefully covered with a small round coverslip. During this process the male genital capsule was gently pressed out of the gaster. After drying, the slides were examined at 100 x and 400 x magnification under the light microscope and morphological characters were determined for species identification according to Pintureau (2011). The morphological classification of pupal parasitoids was based on Goulet and Huber (1993) and a detailed description by Smith (1932).

3 RESULTS

3.1 ECB flight and parasitation levels

The ECB pressure varied between northeast Slovenia (Rakičan) and central Slovenia (Jablje). Higher catches were recorded in Rakičan. ECB first occurred in Rakičan at the end of May, early June. In Jablje, ECB was first observed in mid-June. In both localities two ECB flights were observed, although the two-flight trend was more evident in Rakičan. The first ECB flight peaked from 17-20 June, and the second from 15-20 July. In 2012 in Jablje the first ECB flight was not very distinct. ECB flight waned in August (Figure 1).

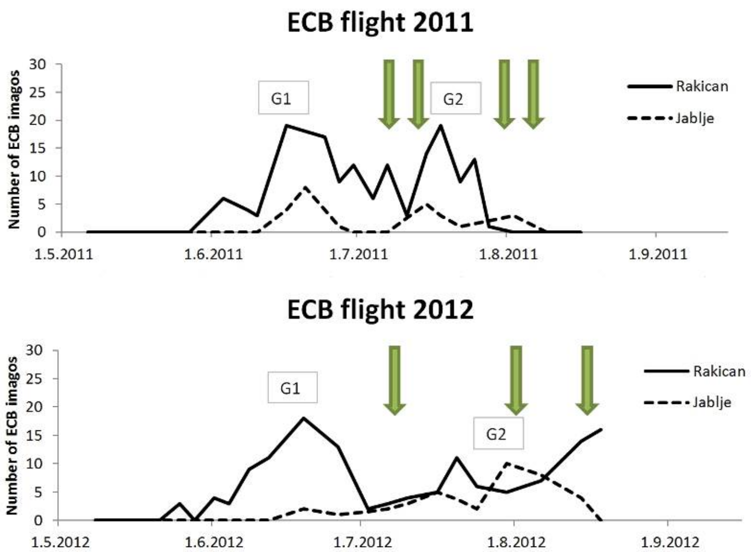

Figure 1: ECB flight in Jablje and Rakičan in 2011 2012. Green arrows show approximate times of ECB egg mass and pupae sampling. *G1* or *G2 – first or second ECB flight.*

During the five years of the survey over 3000 maize plants were inspected for the presence of second generation ECB egg masses. Altogether 84 ECB egg masses were discovered. Five of these 84 egg masses were parasitized, resulting in a percentage parasitism of nearly 6 %. Approximately 150 ECB-damaged plants were dissected to obtain potentially parasitized second generation ECB pupae. Approximately 30 pupae were transferred to the laboratory. Two of them were harbouring parasitoids (percentage parasitism: nearly 7 %). The egg masses were parasitized by *Trichogramma brassicae* Bezdenko, 1968, whereas the two parasitized pupae were parasitized by *Tycherus nigridens* Wesmael, 1845. Taxon *T. nigridens* had not been reported previously in Slovenia according to our knowledge.

3.2 *Trichogramma brassicae* Bezdenko, 1968

The parasitized ECB egg masses were typically discovered on the abaxial side of 3rd or 4th maize leaf from stem base up. ECB eggs usually formed aggregates of 20-35 eggs and were located as a single cluster near the central leaf vein. Some parasitized egg masses were completely parasitized, whereas some only partially. After transfer to the lab, minute wasps emerged in 3-5 days from the uniformly black ECB egg masses (Figures 1 and 2). The wasps were minute, ca. 0.9 mm long, yellow or yellow and black / brown with bright red eyes, short antennae, compact bodies and severely reduced wing venation. Based on morphological characteristics described in Cônsoli *et al*. (2010), Ferriere and Kerrich (1958), Goulet and Huber (1993), they were classified to the genus *Trichogramma*. The genus *Trichogramma* contains more than 200 described species (Pinto, 2006). Mostly they are generalists parasitizing lepidopteran, dipteran, neuropteran and coleopteran egg masses (Querino *et al*., 2010).

They are commonly used as an alternative measure to control major agronomical pests via inundative releases (Kölliker-Ott *et al*., 2004). For more detailed classification to the species level, Pintureau (2011) was used. Based on morphological characteristics of male genitalia the emerged *Trichogramma* wasps were classified as *Trichogramma brassicae* (Figure 3). The hymenopteran parasitoid *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) was introduced into Western Europe to control ECB more than 40 years ago (Babendreier, 2003b). The introduced strain originates from Moldavia. Initially it was described under the name *T. maidis* Pintureau and Voegelé (Pintureau and Voegelé, 1980) and later assigned to be the neotype of *T. brassicae* Bezdenko by Pintureau (1987). Its present day areal extends throughout European mainland, including, but not only, Austria, Belgium, Bulgaria, Germany, France, Italy, Moldova, Romania, Spain, Switzerland, The Netherlands and Ukraine (Fauna Europea, http://www.faunaeur.org/, accessed on June 4th, 2015). The species develops idiobiontically inside the egg of the host (Boivin, 2010), where it also overwinters (Stengel *et al*., 1977).

Figure 1: *Ostrinia nubilalis* (ECB) non-parasitized hatching egg mass (left). Parasitized ECB egg mass (right). Scale bar on the right picture – 1.0 mm. Photos: Š. Modic.

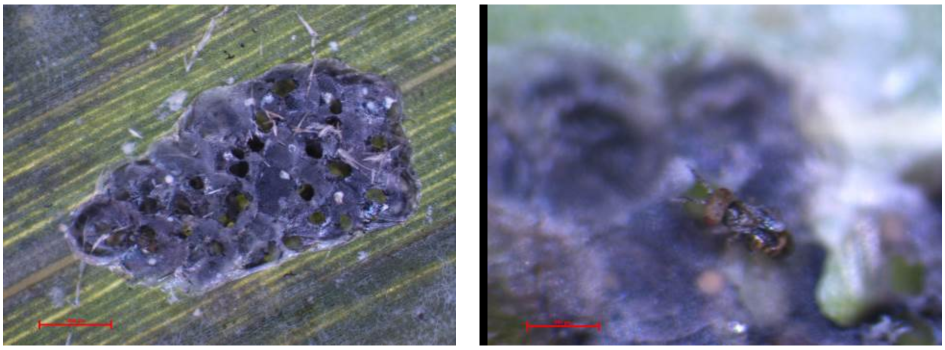

Figure 2: *Ostrinia nubilalis* parasitized egg mass with *Trichogramma brassicae* emergence holes (left). Freshly emerged *Trichogramma brassicae* female (right). Scale bar represents 1.0 mm on the left and 0.5 mm on the right picture. Photos: J. Razinger.

Figure 3: Left picture: *Trichogramma brassicae* female (left) and male (right). Right picture: microscopic preparation of a *Trichogramma brassicae* male. Scale bar represents 0.1 mm on the left and 0.2 mm on the right picture. Photos: J. Razinger.

3.3 *Tycherus nigridens (Wesmael, 1845)*

The pupae sampled for pupal parasitoid inventarisation were collected from maize plants evidently infested by ECB, exhibiting breakage, holes and / or frass. Most often, the pupae were discovered near the stem base. They were carefully removed from the plants using a Swiss army knife and tweezers. Upon transfer to the lab, the pupae were observed on a daily basis for ECB or parasitoid emergence. When an ECB emerged it was discarded. The emerged parasitoids were classified according to Goulet and Huber (1993) and Smith (1932) to *Tycherus nigridens*. *T. nigridens* (Wesmael, 1845) (Hymenoptera: Ichneumonidae; synonym: *Tycherus planifrons* Wesmael, 1845, *Phaeogenes nigridens* Wesmael) is an internal solitary parasite which attacks the pupal stage of ECB (Smith, 1932). It prefers 1- and 2-day old pupae, which it perforates with its ovipositor and lays the eggs free in the body cavity of the host (Baker *et al*., 1949). The earliest definite reference to this species in literature appeared in 1844, when Wesmael first described it as *Phaeogenes nigridens*. In addition to the corn borer the only other host from which *Tycherus nigridens* has been reared is *Tortrix pronuboena* Hübner (Smith, 1932). The adult is distinguished from the other parasites of ECB by its robust appearance and its rusty red-brown abdomen with black terminal portion. The larva has at least four, maybe five, distinct instars. The adults have long life spans; some females were kept alive at low temperature for 10 months (Smith, 1932). It overwinters as an adult female (Baker *et al*., 1949). Originally described from Belgium, this species was initially reported from Sweden, Germany, Spain and France (Smith, 1932). Its present day areal includes most of the EU countries, with the exception of the following countries for which no data exists: Albania, Belarus, Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Iceland, Ireland, Kosovo, Latvia, Lithuania, Macedonia, Moldova, Montenegro, Portugal, Serbia, Slovakia, Ukraine and Vojvodina (Fauna Europea, http://www.faunaeur.org/, accessed on June 4th, 2015).

Figure 4: *Tycherus nigridens* (left) and a detail of forewing venation (right). Scale bar represents 1.0 mm on both pictures. Photos: J. Razinger.

4 DISCUSSION

During the five years of the systematic survey we discovered two ECB parasitoid species. ECB egg masses were parasitized by *Trichogramma brassicae*, already reported in Slovenia by Bohinc *et al*. (2015), whereas ECB pupae were parasitized by *Tycherus nigridens* (new finding for Slovenia), with 6 or 7 % parasitation rate, respectively. More effort was invested in searching for egg mass parasitoids (> 3000 plants examined). Thus, to be sure of the exactness of *T. nigridens's* observed natural parasitation rate of 7 %, we would need to sample a much greater number of pupae.

Trichogramma occurs in all vegetated terrestrial habitats that have been sampled in all six biogeographic regions: Palearctic, Oriental, Nearctic, Neotropical, Afrotropical and Australasian (Querino *et al*., 2010). *T. brassicae* was isolated from several host species (Pintureau, 2011). In Slovenia it was first discovered on August 18, 2014, on *Mamestra brassicae* (Linnaeus, 1758) eggs (Bohinc *et al*., 2015). However, due to their small size, species of *Trichogramma* can potentially be transported by wind and intentionally or otherwise, by man from one country to another in a short period of time. Because of this it is often difficult to determine individual species' natural range of distribution (Querino *et al*., 2010).

Attempts at classical biological control of ECB employing *Tycherus nigridens* release were performed already in 1924 in the USA when a single colony was released in Massachusetts. Attempts intensified by releasing more numerous parasitoids imported from Europe and Japan, totalling more than 50.000 *T. nigridens* between years 1926-1933 (Baker *et al*., 1949). In their report Baker *et al*. (1949) conclude that the biocontrol program was successful as entomologists were able to recover *T. nigridens* in localities where it was not released in the previous three years, but that more knowledge on the biology of the parasitoid would be needed in order to improve the storage conditions of the adults enabling better synchronization of the releases so the parasitoid would establish broader. Smith *et al*. (1932) reported that *T. nigridens* was the most effective ECB parasite in Europe with the maximum parasitism rate of 17.5 % recorded in 1930 in the fields around Padova in northern Italy. Factors that may limit its effectiveness as an effective biocontrol agent are the small number of eggs produced by each female; the mortality of some ovarian eggs, especially among hibernating females; the rather long oogenetic period; and the generally unsuitable oviposition conditions in the spring (Smith *et al*., 1932). Two related *Tycherus* species are reported in literature as pupal parasitoids of agricultural or forestry pests: cherry bark tortrix, *Enarmonia formosana* (Scopoli, 1763), an invasive orchard pest in northwest US, is parasitized by *Tycherus vagus* (Berthoumieu, 1899) (Jenner *et al*., 2004), whereas *Cydia strobilella* (Linnaeus, 1758), a Holarctic pest of spruce cones, is parasitized by *Tycherus fuscibucca* (Berthoumieu, 1901) (Brockerhoff and Kenis, 1996).

T. nigridens represents a new taxon report for Slovenia. However, this taxon has been previously reported from many EU countries, including some of Slovenia's neighbouring countries like Austria, Italy and Hungary, but not Croatia. This fact reveals the problem of reliability of such taxon distribution information, as the real, biological distribution is probably underestimated due to insufficient funding allocated to natural enemy inventarisation studies, especially in countries with a lower gross domestic product per capita. In line with the present Slovenian legislature (Official Gazette of the RS, 83/12, 36/10 40/14, 62/07 and 45/06), potential introduction of commercially reared natural enemies and their release can only be done after obtaining concrete evidence on the autochthonous presence of these species as the pests' natural enemies. Thus our study clearly illustrates the need for undertaking systematic surveys of natural enemies of agricultural pests, so that concrete evidence for their autochthonous presence can be confirmed and their introduction possible.

Specific risks exist when releasing large number of Lepidopteran egg parasitoids. Laboratory experiments demonstrated that *T. brassicae* can attack many non-target butterflies including endangered species (Babendreier *et al*., 2003a). However, non-target butterflies were very seldom attacked under natural conditions in Switzerland (Babendreier *et al*., 2003b). Besides having adverse effects on non-target organisms, inundatively released non-indigenous *Trichogramma* may compete with locally occurring *Trichogramma*. Therefore preference should always be given to indigenous strains or species, when developing a new IPM program based on *Trichogramma* (Herz *et al*., 2007).

Despite the mentioned risks, one should consider the benefits of parasitoid-based biological control strategies. The released parasitoids do not harm autochthonous population of generalist predators and other beneficial arthropods (Chapman *et al*., 2009; Zhang *et al*., 2010); the application has no negative effect on farmers' health; the environment, especially the ground- and surface water is not burdened by pesticides and their breakdown or transformation products (Zhang *et al*., 2010), and the food or feed is free from insecticide residues.

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