Originalarbeit

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First records of the Z-Race of European Corn Borer *Ostrinia nubilalis* (Hübner 1796) from Scandinavia

Erster Nachweis der Z-Rasse des Maiszünslers *Ostrinia nubilalis* (Hübner 1796) in Skandinavien

Abstract

European Corn Borer (ECB, *Ostrinia nubilalis*) is among the main pests of maize in Europe and throughout its introduced range. Two morphologically indistinguishable pheromone races of the species exist, the so-called E-race and Z-race. In Germany the E-race is most common in mugwort (*Artemisia vulgaris*) whereas the Z-race occurs mainly in maize and is responsible for the main proportion of the economic damage. But both races have several alternative host plants. For example, the E-race can also occur in maize. The species has been known from Scandinavia already for a considerable time period, but no occurrence in maize was reported until recently. In 2010 larvae were found in maize at one site on the isle of Sealand, Denmark. A monitoring with Z-race pheromone traps in Denmark in 2011 did not result in any catches of ECB. In the same year however, ECB larvae were found in maize at 3 sites in southern Sweden. Larvae from 2 sites were assigned to Z-race via PCR. Independently from these finds, adult moths caught on the isle of Gotland in 2010 were also confirmed to be Z-race by PCR. This is the first evidence of the occurrence of the Z-race in Scandinavia and the northernmost find of the Z-race in Europe so far. Implications for plant protection are discussed.

Key words: European Corn Borer, Scandinavia, Z-race, pheromone, fatty acyl reductase

Zusammenfassung

Der Maiszünsler (*Ostrinia nubilalis*) ist einer der bedeutendsten Schädlinge im Mais in Europa und in den Gebieten seiner Einschleppung. Er tritt in 2 Pheromonrassen (E und Z) mit in Mitteleuropa derzeit unterschiedlichen Hauptwirtspflanzen auf. Die E-Rasse ist häufig in Beifuß (*Artemisia vulgaris*) zu finden, während die hauptsächlich im Mais auftretende Z-Rasse in Deutschland den ökonomisch bedeutenden Schaden verursacht. Beide Rassen können jedoch auch andere Pflanzen befallen. So kann beispielsweise die E-Rasse auch am Mais auftreten. Eine morphologische Unterscheidung der Rassen ist nicht möglich. Für Skandinavien lagen bisher zwar Daten über das Vorkommen des Maiszünslers vor, jedoch keine Berichte über das Auftreten in Mais. In 2010 wurden Maiszünsler-Larven an einem Standort im Mais auf der Insel Sjaelland (Seeland), Dänemark, gefunden. Ein Monitoring in Dänemark 2011 mit Pheromonfallen für die Z-Rasse erbrachte keine weiteren Funde. 2011 wurden jedoch in Südschweden am Mais an 3 Standorten Befall durch Maiszünsler-Larven festgestellt. Mittels PCR wurden Larven von 2 Standorten untersucht und als zur Z-Rasse gehörig bestätigt. Unabhängig davon wurden in 2010 Falter von der schwedischen Ostseeinsel Gotland ebenfalls als Tiere der Z-Rasse bestätigt. Dies sind die ersten Nachweise der Z-Rasse für Skandinavien und in Europa bislang die nördlichsten belegten Funde dieser

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Rasse. Implikationen für den Pflanzenschutz werden diskutiert.

Stichwörter: Maiszünsler, Skandinavien, Z-Rasse, Pheromon, Fettsäure-Reduktase

Introduction

The European Corn Borer (ECB, *Ostrinia nubilalis*) is among the main pests of maize in Europe and throughout its introduced range in North America, but is not restricted to this crop. The polyphagous ECB thrive on almost any herbaceous wild or cultivated plant with stems large enough for the larvae to enter and bore (Hudon and LeRoux, 1989). A major host criterion is apparently a stem thick enough to tunnel into, with survival being higher in thicker stems (Losey et al., 2002). Two morphologically indistinguishable pheromone races of the species exist: the so-called E- and Z-race (e.g. Lassance, 2010). At least in middle Europe, the two pheromone races may exhibit different host plant preferences. The Z-race feeds primarily on maize and can cause high economic damage to this crop. Other crops, vegetables and ornamentals may also be attacked. On the other hand, the E-race is often associated with mugwort (*Artemisia vulgaris*) and hop (*Humulus lupulus*); apart from these, it also attacks over 200 plants from different families and is said to account for most of the wide host range (Langenbruch, 2007; Caffrey and Worthley, 1927). The E-race may also attack maize, like it does in a limited part of North America. However, in middle Europe, the E-race has colonized this crop only sporadically. Hence, the main economic damages in middle Europe are imputable to the Z-race.

The distribution of the two races in middle Europe is far from being clear and needs more detailed characterization. Records published (Anglade et al., 1984; Peña et al., 1988) should be updated, as both races seem to be in the process of range expansion. In the UK, the species is considered a continental European species that has become established in the south since the 1930 s with larvae apparently feeding on mugwort (Goater, 1986) and hop (Lassance et al., 2011). Pheromone gland analysis indicates the race associated with these host plants is the E-race. In 2010 however, ECB damage and larvae in maize were found at a site in the Southwest of England (Korycinska and Cannon, 2010). In France, the ECB is found on maize throughout the country, whereas it seems to occur on mugwort only in the northern part (Martel et al., 2003).

In the northern half of Germany, the species was reported at a relatively early point (Heddergott, 1977; Krause, 1978; Palm, 1986), but generally in mugwort and not in maize. During a short period from 1983–1985 the species also occurred in maize near Recklinghausen (Ruhrgebiet) (Welling and Langenbruch, 1984), but always in close connection to stands of mugwort. Using pheromone trapping, the ECB attacking maize in this single case were identified as E-race (Langenbruch et al., 1985). At that time the northernmost occurrence of the Z-race in Germany was around Koblenz (Langenbruch et al., 1985). This demonstrates the ability of the E-race to occasionally colonize maize, but maize in Germany is almost exclusively used by the Z-race (Lorenz, 1993). Today in Germany maize-inhabiting populations of ECB are closely monitored to follow their northward spread. To date, they have already been recorded in the three northernmost federal states, which are Mecklenburg-Western Pomerania, Lower Saxony and Schleswig-Holstein. Moreover, in the eastern part of Mecklenburg-Western Pomerania, the Baltic Sea coast has been reached (http://www.agrar.de/de/tl_files/karten/zuensler_800.jpg). Gaspers (2010) confirmed that ECB collected on maize in Germany, France, Spain, Serbia and Croatia could be assigned mainly to the Z-race. No long-term adaption of the E-race to maize was observed in Germany so far. This is contrary to the situation in some southern European countries (e.g. Italy, Greece) where maize is attacked by the E-race (Gaspers, 2010).

Occurrence of *Ostrinia nubilalis* in Scandinavia

As the northernmost part of the species range in Europe, Scandinavia did not receive much attention, with the amount of maize grown being comparatively low and no ECB damages being recorded. But ECB has been observed in Scandinavia already for a considerable time period. On 13th July 1958 the first specimen for Norway was found around Sogne at the southern tip of the country. This individual was considered to be a migrant (Aarvik, 1989). The species occurs more frequently in Denmark, Sweden and Finland. Here it is also partly a migrant, but resident populations occur already in eastern Denmark and southern Sweden. In Denmark it is even fairly common on Bornholm, the eastern parts of Sjaelland, Falster and Moen (Palm, 1986).

Apart from mugwort, known food plants of the larvae in Denmark are for example various species of dock (*Rumex* spp.) (Palm, 1986). No information on the race is given but the food plants reported suggest that the race observed may have been the E-race. According to Lorenz (1993) in Germany the post diapause temperature requirements of the E-race were much lower than those of Z-race, even for sympatric E-race and Z-race populations. As a consequence, E-race individuals emerged several weeks earlier than Z-race individuals. The lower temperature requirements of the E race may make the occurrence of this race in Scandinavia more likely. No observations of larvae in maize were reported from Scandinavia prior to 2010.

Recent situation in Denmark

A map of recent observations of adult ECB moths is available for Denmark 2007–2011 (http://www.fugleognatur.dk/artintro.asp?ID=2150). Details of these recent observations are also available (http://www.fugleognatur.dk/art2.aspx?mode=obs&id=2150). In September 2010, unknown Lepidoptera larvae were found in about 8–10 corncobs in

a 5 ha field of sweet corn at Boeslunde, Sealand, Denmark, and tentatively identified as ECB. These larvae were only detected because all corncobs from this field were inspected for pests or damages before being packed for the market. Therefore the location of the infestation within the field remained unknown.

The corncobs with the larvae were sent to the Julius Kühn-Institut (JKI), where the identification as ECB was confirmed. The race involved was not investigated at that time. Following the find in 2010, the Danish Knowledge Centre for Agriculture performed a monitoring of ECB at 22 sites distributed over the whole country of Denmark in 2011. The traps used in the monitoring were CORETRAP pheromone traps by Riff 98 soc. coop. r.l baited with the Z-pheromone. Catches were identified at Danish Knowledge Centre for Agriculture, Crop Production, and at JKI. No ECB were found in any of these traps from any Danish site in 2011.

Recent situation in Sweden

A map of recent observations of adult ECB moths is available for Sweden 2011 (http://www2.nrm.se/en/svenska_fjarilar/o/ostrinia_nubilalis.html). Details of these recent observations are also available (http://www.artportalen.se/bugs/bugs_gallery.asp?artid=215494). During August 2011, unknown Lepidoptera larvae were found feeding on maize in two maize fields located at opposite coasts of Skane, the southernmost Swedish province, and tentatively identified as ECB. The sites were Landskrona near Lund and Ravlunda close to Simrishamn. At Landskrona, several larvae were found in a small patch of the field early in August. At Ravlunda only few larvae were found in mid-August and at the end of August. At both these sites, the larvae were discovered deep in the field, far from the field edge. In early September 2011 a few larvae were discovered in a third field about 150 km further northeast on the isle of Öland, east of Kalmar. In contrast to the situation at Landskrona and Ravlunda the larvae occurred on maize close to the field edge. These larvae were also identified as ECB.

5 larvae from Landskrona and 2 larvae from Ravlunda were sent to JKI where the identification as ECB was confirmed. To clarify which race occurs in maize in Sweden, 3 larvae from Landskrona and 2 larvae from Ravlunda were subsequently sent to the Department of Zoology, Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences. All 5 larvae turned out to belong to the Z race. This result was evidenced by PCR, using the specific primers for the E race-specific and the Z-race-specific fatty acid reductase genes (FAR-E and FAR-Z, described by Lassance et al. (2010). For *pgFAR*, pgFAR-E_forward: 5'-GGTTTGATATTGATTGAGGAGAG-3'; pgFAR-E_reverse: 5'-GGTTTGTTTGGTTGTAATTTATAGG-3'; pgFAR-Z_forward: 5'-CGACTAGAGTAGGTATGTAATATAG-3' and pgFAR-Z_reverse: 5'-TTGAGTAAGCGTTTGTATGAAG-3' primers were used. The presence of the Z-race-specific *pgFAR* gene was confirmed by PCR using genomic DNA isolated from larvae with the REDExtract-N-Amp PCR kit (Sigma) following the manufacturer's instructions. PCR amplification used 1×PCR buffer and 0.5 unit Taq DNA polymerase (KAPA Biosystems), 4 µL of extract, 0.4 µM of primers, 200 µM of each dNTP and 2 mM $MgCl_2$ in a final volume of 25 µL. Standard PCR cycling conditions were one cycle at 94°C for 3 min, and 30 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min followed by a final extension of 72°C for 10 min. To confirm the identity of the amplified cDNA fragments, we separated 10 µl aliquots of the PCR products on a 2% agarose gel containing 1×GelRed nucleic acid stain (Biotium) followed by detection with an Alpha-Imager EP fluorescence imaging system (Alpha Innotech Corp., San Leandro, CA, USA). Genomic DNA extracted from adult females coming from an E- and a Z-strain of *O. nubilalis* laboratory cultures, maintained in strict separation in the Hungarian laboratory, respectively, served as reference samples. Strain identities of these cultures were determined by analysing their respective pheromones by means of gas chromatography equipped with a simultaneous electroantennographic detector (GC-EAD), as described earlier (Kárpáti et al., 2007; Király and Szőcs, 2009).

At the same time it became apparent that an earlier, but yet unpublished record of the Z-race from a different location in Sweden existed. Already in 2010, 7 adult specimens of ECB were collected by Lars Petterson (Department of Biology, Lund University) during a biodiversity inventory on the isle of Gotland. Affiliation to race was performed by JML at the Department of Biology, Lund University, Sweden. Briefly, genomic DNA was extracted from the dried specimens using the DNeasy tissue kit (Qiagen, Sollentuna, Sweden) and used as template for PCR amplification of the molecular markers *pgFAR* (Lassance et al., 2010), *Onub-OR1* and *Onub-OR3* (Lassance et al., 2011). For *pgFAR*, exon 5 and exon 7 were amplified using specific primer pairs (pgFAR_E5_forward: 5'-ATCTATGTCTCTAGCGCATA-3'; pgFAR_E5_reverse: 5'-TAAAAAAATATCCRTWAKTCGTG-3'; pgFAR_E7_forward: 5'-TCTTCGATTCGGGAACCCATA-3'; pgFAR_E7_reverse: 5'-TCTTTCRTTGGATTCCCATGC-3'), whereas *Onub-OR1* and *Onub-OR3* were amplified using primers reported in Lassance et al. (2011). All PCR reactions and purification and sequencing products were performed using the conditions described in Lassance et al. (2011). For all 7 specimens analysed (3 males and 4 females), the sequences of all markers were in agreement and unequivocally revealed the moths as pure Z-race individuals (Fig. 1, Fig. 2, Fig. 3).

Discussion

From 2010 to 2011, European Corn borer (*Ostrinia nubilalis*) larvae were discovered in maize at 4 sites in Scandinavia: 1 in eastern Denmark and 3 in southern Sweden. The occurrence of ECB in Sweden and Denmark is well known and not unusual in itself (e.g. Palm, 1986), but the occurrence on maize has not been reported from this region before. Larvae from 2 Swedish sites were analysed for race identification by PCR and were confirmed to

Fig. 1. ECB (*Ostrinia nubilalis* (Hübner) larva in corncob from Boeslunde, Denmark 2010.

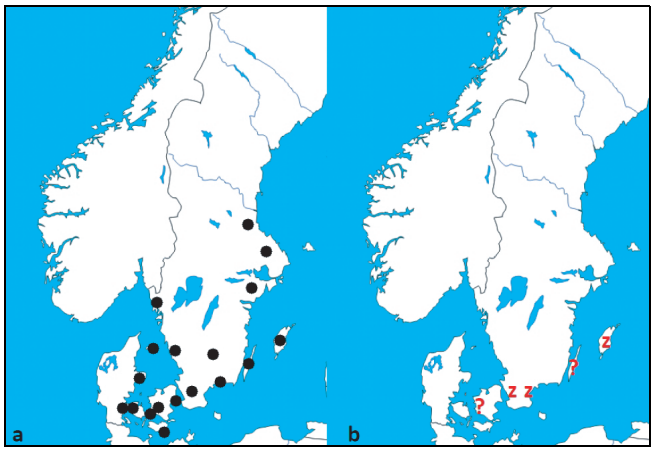

Fig. 2. a) Recent observations of ECB (*Ostrinia nubilalis* (Hübner) adults in Denmark (2007–2011) and Sweden (2011). Modified after http://www.fugleognatur.dk/artintro.asp?ID=2150 and http://www2.nrm.se/en/svenska_fjarilar/o/ostrinia_nubilalis.html; b) finds of larvae in maize or of adults; z = confirmed as Z race (larvae from Landskrona and Ravlunda, adults from Gotland); ? = race unknown (larvae from Boeslunde and Öland).

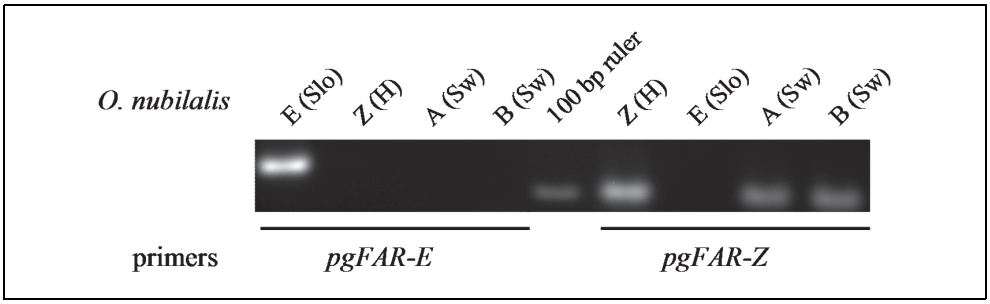

Fig. 3. PCR products of genomic DNA from the E- and Z-race of *O. nubilalis* laboratory culture adult females originated from Slovenia (Slo) and Hungary (H), respectively, and from Swedish larvae. (A, Ravlunda, Sweden; B, Landskrona, Sweden).

belong to the Z-race. Furthermore ECB adults collected in the summer of 2010 on Gotland, were analyzed for race identification and were also confirmed to be Z-race. As the PCR method used would also allow for the detection of hybrids between the races, this possibility can be excluded for the individuals analysed. We conclude that these specimens are the first evidence of the Z-race for the Scandinavian countries and the northernmost confirmed finds of this race. The availability of efficient tools to precisely discriminate the pheromone races has and will allow for the design of optimal control strategies.

For larvae from the Danish site and one Swedish site no PCR could be performed. Therefore in these cases it cannot be excluded that they belonged to the E-race and their occurrence in maize may be accidental. The lack of ECB catches in the Danish monitoring using the Z-pheromone traps could either be due low occurrence of the Z race, or the sometimes low performance of pheromone traps (SZŐCS and BABENDREIER, 2011), or to a colonization of maize by the E-race. No data seem to exist on the pheromone race of the previously known Scandinavian ECB populations. The lower temperature requirements of the E-race (LORENZ, 1993), as well as records of larvae from E-race food plants and no observations of larvae in maize may point to E-race populations, though this is speculative at this point.

The recent finds of the Z-race in Scandinavia in at least 3 locations may have been either due to active spread or assisted by human transport (e.g. ship-assisted). The species itself is known as a migrant with the Z-race expanding its range further north as a consequence of climate change as well as changes in the agricultural practices (PORTER, 1995). For example the first reports of the occurrence of ECB in maize in Lower Saxony are only from 2006, but in that year the species was already detected at 7 sites (KRÜSSEL, 2007). On the other hand ECB has already a history of accidental introduction by humans, e.g. into North America via contaminated broomcorn (CAFFREY and WORTHLEY, 1927).

The findings, especially the occurrence of adults already in 2010, indicate that the Z-race may have been present in Scandinavia for at least two years and that a risk for economical damage exists, even though maize is not a major crop in the Scandinavian countries. Nevertheless, the maize growing area in Denmark has increased from

100 000 ha to 185 000 ha from 2002 to 2011. In Sweden the maize growing area is even smaller, with around 16 325 ha in 2010 and around 15 600 ha in 2011, but in general the area has also been increasing over the last years. However, low infestations of ECB will be difficult to detect and may not cause significant damage. Therefore at the moment the impact on maize growing in Scandinavia is not predictable. With the constant improvement of maize varieties and in particular a decrease in temperature sum leading to a northwards expansion of the suitable range for maize culture, damages of economical significance may be observed in the close future. In Mecklenburg-Western Pomerania and in Saxony, the species abundance and the damage caused have considerably increased since the first observations in maize (PÖLITZ et al., 2007; HEIDEL, 2007). The species occurrence in maize in Denmark and Sweden should be closely observed in the future.

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