Sorghum, Miscanthus & Co: Energy crops as potential host plants of western corn rootworm larvae

Sorghum, Miscanthus *& Co: Energiepflanzen als potenzielle Wirtspflanzen des Westlichen Maiswurzelbohrers*

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Summary

In a series of greenhouse experiments the host status and quality of 49 biofuel plants for the larvae of WCR were evaluated. The plants tested (18 species and varieties of *Sorghum*, 16 forage grasses, 6 *Miscanthus* genotypes, 6 *Panicum* varieties and 3 broadleaf species) were grown for at least three weeks before they were used in the bioassays.

The insects used in the experiments were obtained from a non diapausing laboratory strain originally from the US and maintained by BTL since 2006. Only neonate larvae (not older than 24 hours) were used in the bioassays.

In each experiment up to six species or varieties of plants were tested each with 10 replicates (containers). A susceptible maize variety was used as a positive control in each experiment. Each plant container was infested with ten neonate WCR larvae using a fine art brush. After inoculation the plants were not watered for at least 24 hours to facilitate the establishment of the larvae. The experiments were terminated after 18 days. To extract surviving larvae the soil and roots of test plants were carefully examined by hand and then transferred to a modified MacFadyen heat extractor with an extraction temperature of 45 °C.

To assess the host quality the number of larvae recovered, the widths of their head capsules and dry weights were recorded. The larvae were dried at 40 °C for at least 72 hours and then weighed on an electronic micro balance

Of the 21 forage and switch grasses examined 16 hosted WCR larvae. However, the percentage of larvae that survived for 18 days, their dry weights and head capsule widths were significantly less than that recorded for larvae that developed on maize roots. The roots of most (i.e. 15) of the 18 *Sorghum* species or varieties tested were unsuitable for the development of WCR larvae. For the remaining three *Sorghum* species a maximum of only two larvae (of 100 inoculated) were recovered. These results indicate that species of *Sorghum* are very poor quality hosts for WCR as previously reported in other studies. The opposite was true for the *Miscanthus* species tested. The number of larvae recovered from *Miscanthus* x *giganteus* roots, their dry weight and head capsule widths were the same as those recorded for larvae reared on the maize control. The other *Miscanthus* genotypes were less suitable than Mxg, but still more acceptable than all the forage and switch grasses tested in this study. In accordance with all previous studies, which used host plants other than maize, no larvae developed on the three broad leaf species.

Keywords: *Diabrotica virgifera virgifera*, field grasses, energy-crops, recovery, dry weight, head capsule width

Zusammenfassung

In Gewächshausversuchen wurde die Wirtspflanzeneignung von 49 Pflanzenarten und –sorten für Larven des Westlichen Maiswurzelbohrers einer nicht diapausierenden Linie des USDA untersucht. Getestet wurden Pflanzen, die interessante Optionen für die Biomasse-Produktion darstellen. Es wurden 18 *Sorghum*-Hirsen, 16 Ackergräser, jeweils sechs Rutenhirsen und Chinagräser, sowie drei zweikeimblättrige Pflanzenarten untersucht. Die Einschätzung der Wirtsqualität der überprüften Pflanzen erfolgte anhand der Wiederfunde der eingesetzten Larven, deren Kopfkapselbreite und des erreichten Gewichts.

Alle getesteten *Sorghum*-Arten und -Sorten wiesen keine, oder nur eine minimale Wirtseignung für *Diabrotica*-Larven auf. Die getesteten Hirsen können auf Basis dieser Ergebnisse uneingeschränkt als Mais-Alternative im Energiepflanzenanbau empfohlen werden. In einer wechselnden Fruchtfolge mit Mais bieten sie die Chance, hohe Biomasseerträge mit einer effektiven Reduktion der Populationsdichte zu verbinden.

Die Wirtspflanzeneignung der geprüften Ackergräser ist dagegen arten- und sortenabhängig. Fünf der 16 getesteten Gräser stellten sich für die Entwicklung der Larven als ungeeignet heraus. Die Wirtspflanzenqualität der elf Testpflanzen, von denen Tiere extrahiert wurden, muss auf Basis der erhobenen Versuchsparameter als reduziert

bis minimal eingestuft werden. Im Vergleich zur Maiskontrolle waren insbesondere die Wiederfunde, aber auch die Kopfkapselbreiten und das erreichte Gewicht signifikant kleiner. Gleiches gilt auch für die geprüften *Panicum*-Sorten. Eine der sechs überprüften Sorten eignete sich nicht als Wirtspflanze für die Larven und die anderen fünf in nur sehr reduziertem Maße. Diese Ergebnisse zeigen, dass diese Gräser in der Fruchtfolge nicht als Ersatz für Mais bei Eradikationsbemühungen punktuell auftretender Käfer-Populationen zu empfehlen sind. Da die Entwicklungsbedingungen für die Larven aber stark reduziert sind, können in *Diabrotica*-Eingrenzungsgebieten sowohl Ackergräser als auch Rutenhirsen erfolgreich in wechselnden Fruchtfolgen mit Mais eingesetzt werden.

Die einzige Testpflanze an der sich *Diabrotica*-Larven genau so gut wie an Mais entwickelten, ist das Chinagras (*Miscanthus* x *giganteus*). Die anderen fünf getesteten *Miscanthus*-Genotypen ermöglichten ebenfalls eine Entwicklung der Larven. Die Wirtspflanzenqualität dieser Genotypen ist jedoch geringer als von *M.* x *giganteus*.

Stichwörter: *Diabrotica virgifera virgifera*, Ackergräser, Energiepflanzen, Wiederfunde, Trockengewicht, Kopfkapselbreite

1. Introduction

One of the most important biofuel crops is maize. To reduce the negative effects of large scale and continuous cultivation of maize a number of other species of potential biofuel plants have been suggested. Although there are a considerable number of studies on the economic, environmental and social aspects of their cultivation, the direct or indirect effects of these plants on agricultural pests are rarely investigated (Spencer and Raghu, 2009). In particular, whether these plants are suitable hosts for the larvae of the western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, is unknown and will be analyzed in this study.

Reports from the USA indicate that *Diabrotica* larvae not only feed on maize, but can reach the adult stage also on other monocotyledonous plants (Branson and Ortman, 1967a, b, 1970; Oyediran et al., 2004; Wilson and Hibbard, 2004). Some of the plants tested in the USA, other than weeds and wheat, also occur in Germany. Moeser (2003) reports that *Diabrotica* larvae develop as well on wheat and some other species of grass (*Setaria* spp. and *Panicum milliaceum*) as on maize. Unfortunately these experiments were only done using second instar larvae and over a period of only six days. Therefore, it is uncertain whether they can complete their development and reproduce feeding on these grasses.

Field experiments in Romania have confirmed that this beetle will feed on three species of *Setaria* in the field (Breitenbach *et al*., 2005). In contrast to the findings of Moeser and Vidal (2004) no adults were found on wheat in Romania. Cabrera Walsh (2007) report that a species of *Diabrotica* (*D. speciosa* [Germar]) from South America can also complete its development on *Sorghum halepense*. This is of special interest as *Sorghum* spp. are thought to be no suitable host plants for WCR because the roots contain high levels of hydrocyanic acid (Branson *et al*., 1969). As in previous studies only a small number of *Sorghum* plants were analyzed but *Sorghum spp.* could be used for biofuel production instead of maize as a consequence these plants were the main focus of this project.

2. Methods

In a series of greenhouse experiments the host status and quality of 49 biofuel plants and a cultivar of maize for the larvae of WCR were compared. The biofuel plants tested (16 forage grasses, 6 *Panicum* varieties, 18 species and varieties of *Sorghum*, 6 *Miscanthus* genotypes, and 3 broadleaved species) were potted in a 1:1 (w/w) mixture of a commercial compost and sandy field soil previously sieved and heat steamed for four hours. The containers used were 1 liter plastic pots. The drainage holes in the containers were closed with a fine stainless steel mesh (mesh size 100 µm) to prevent the escape of *Diabrotica* larvae. Plants were grown for at least three weeks before they were used in the bioassays. The plants were cultivated and experiments done in a greenhouse kept at 22 ± 2 °C, 65 ± 15% RH and a 16 hour photoperiod. Additional light was provided by 400 W Philips Son-T Agro high pressure sodium vapour lamps if the ambient light intensity fell below 10,000 lux. The plants were watered as necessary, no additional fertiliser was added.

Insects – The insects used in the experiments were obtained from a non-diapausing laboratory strain originally from the US and maintained by BTL since 2006. The non-diapausing strain was se-

lected in the USA at the end of the 1960s over a period of 9 generations and since then kept at the Northern Grain Insect Research Laboratory (USDA-NGIRL), Brookings, USA, and produces approximately 6 generations per year (Branson, 1976). The rearing methods applied by us are those described by Branson *et al*. (1975, 1988). Only neonate larvae not older than 24 hours (Fig. 1) were used in the bioassays (Fig. 2).

Fig. 1 *D. virgifera virgifera*: **A** – developmental stages of larvae (L1–L3) and **B** – typically shortened and bent pre-pupa in the earthen cell in which it pupates.

Abb. 1 D. virgifera virgifera*: **A** –Entwicklungsstadien der Larven (L1–L3) und **B** – Typisch verkürzte und gebogene Vor-Puppe in ihrer Puppenhöhle.*

Fig. 2 Design of trial for testing host quality using *Sorghum bicolor* (Arlys, Biomass 150), *S. bicolor* x *sudanense* (Green Grazer), *S. sudanense* (Akklimat) and maize (Tassilo) as the control.

Abb. 2 *Versuchsdesign zur Testung der Wirtsqualität von* Sorghum bicolor *(Arlys, Biomass 150),* S. bicolor *x* sudanense *(Green Grazer),* S. sudanense *(Akklimat) und Mais (Tassilo) als Kontrolle.*

Bioassays – The proof of acceptability of plant species and cultivars was done in a series of successive experiments. In each experiment up to six species or varieties of plant were tested each with 10 replicates (containers). A susceptible maize variety (Tassilo, kindly provided by KWS) was used as a positive control in each experiment. The experiments were set up in a randomized block design. As according to Chege *et al*. (2005) and Hibbard *et al*. (2008), the quality of host plants depends on their age the test plants were at a susceptible vegetative developmental stage (e. g. maize and *Sorghum* spp. at BBCH 14–15, field grasses at the tillering stage, BBCH macro stadium 2; BBCH working group, [2001]). Each plant container was infested with ten neonate WCR larvae using a fine art brush (size 000). After inoculation the plants were not watered for at least 24 hours to facilitate the establishment of the larvae. According to the quarantine rules the experiments were terminated after 18 days, before the animals reached the pre-pupal stage.

To extract surviving larvae the soil and roots of test plants were carefully examined by hand and then transferred to a modified MacFadyen heat extractor (MacFadyen, 1961). Heat extraction was continued for up to four days with an extraction temperature of 45 °C provided by red light bulbs (Fig. 3).

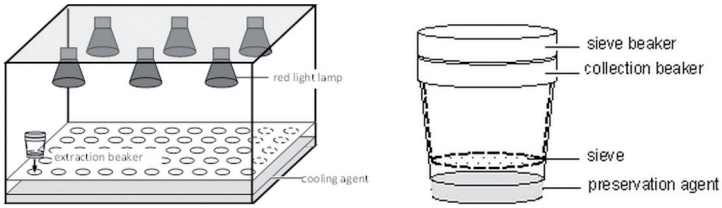

Fig. 3 A – Design of the modified MacFadyen apparatus; B – Extraction beaker.

Abb. 3 *A – Design des modifizierten MacFadyen Apparates; B – Extraktionsbecher.*

To assess the host quality the number of larvae recovered, the widths of their head capsules and dry weights were recorded. Head capsule measurements were obtained using a stereo microscope (Olympus, SZX 12) fitted with a CCD camera (Olympus, colour view III) and cell^D software was used to analyze the pictures (Olympus Deutschland GmbH, Hamburg, Germany and Olympus Soft Imaging Solutions GmbH, Münster, Germany). The larvae were dried at 40 °C for at least 72 hours and then weighed on an electronic micro balance (XP 26, d=0.1 µg; Mettler-Toledo GmbH Giessen, Germany).

Statistics – The number of larvae recovered was expressed as percentage recovered per container (n_{ex}*100/n_{ino}; n_{ex}=number larvae extracted; n_{ino} number of larvae inoculated). For the calculation of the mean head capsule width or dry weight the value for each larva was treated as a replicate. Comparisons of experimental treatments were done using a t-Test or Mann-Whitney Test. To compare more than two treatments a one-way ANOVA and Tukey post hoc test were performed. To test if the preconditions for parametric statistics (normality and equal variances) were met the D'Agostino and Pearson omnibus normality- and Bartlett-test were used. Non parametric comparisons were done using the Kruskall-Wallis test. For all calculations Microsoft Excel® 2002 (10.6871.6870; SP 3) and GraphPad Prism 5.03 for Windows (GraphPad Software Inc., San Diego, USA) were used.

3. Results

A comparison of the results for all the maize controls in the successive experiments shows that the development of larvae was dependent, but not exclusively so, on food quality (Fig. 4). There are significant differences between recovery (ANOVA, Tukey post-hoc test, $F=6.572$, $df=13$; $p< 0.0001$), dry weight (Kruskal-Wallis- and Dunn post-hoc-test, $H=286.3$, $df=13$, $p< 0.0001$) and head capsule width (Kruskal-Wallis- and Dunn post-hoc-test, $H=109.0$, $df=13$, $p< 0.0001$). It is difficult to explain these differences as in each experiment the relevant abiotic and biotic factors (e. g. temperature, soil composition, air- and soil-temperature, duration of experiment, plant age, number of larvae per container) were identical. It cannot be excluded that there are rhythms in the development of beetles and plants. These variations document the accurateness and thresholds of the executed experiments and should be accounted for in the interpretation of the results. The data for each experiment were calculated relative to those for the maize control (=100%) in order to compare the results of the different experiments.

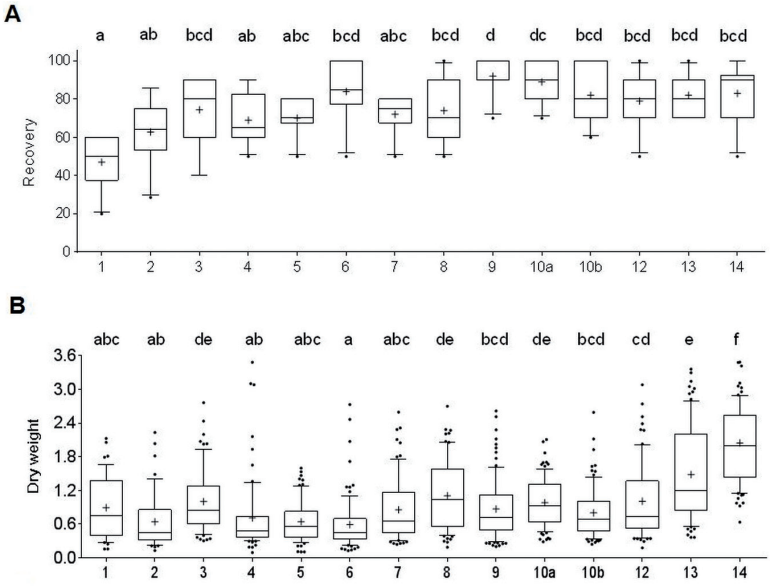

Fig. 4 Results of the individual successive tests in which was analysed (A) percentage recovery, (B) dry weight (mg) and (C) head capsule width (µm) of *D. virgifera virgifera* larvae reared on maize**.** (boxplots with median, mean (‚+') and 10–90 percentiles; different letters indicate significant differences (**A**: one-way ANOVA, Tukey-test; **B** and **C**: Kruskal-Wallis Test, Dunn's-Test; p<0.05).

Abb. 4 *Ergebnisse der einzelnen aufeinander folgenden Versuche, in denen analysiert wurde (A) prozentualer Anteil an Wiederfunden, (B) Trockengewicht (mg) und (C) Kopfkapselbreite (µm)* von D. virgifera virgifera *Larven die an Mais gehalten wurden (boxplots mit Median, Mittelwert (‚+') und 10–90 Perzentile; unterschiedliche Buchstaben kennzeichnen signifikante Unterschiede (**A**: one-way ANOVA, Tukey-Test; **B** and **C**: Kruskal-Wallis Test, Dunn's-Test; p<0,05).*

The survival and development of WCR larvae feeding on 49 plant species/varieties, including 16 forage grasses (e.g. a number of *Lolium* and *Festuca* varieties), six switch grass varieties (*Panicum virgatum*), 18 *Sorghum* species/varieties, *Miscanthus* x *giganteus* (Mxg) and five other *Miscanthus* genotypes and three broadleaved species were monitored. To assess the quality of these plants as hosts for WCR the number of larvae that survived and the widths of their head capsules and dry weights were recorded.

Of the 21 forage and switch grasses examined 16 hosted WCR larvae (Tab. 1 and 2). However, the percentage of larvae that survived for 18 days, their dry weights and head capsule widths were significantly less than that recorded for larvae that developed on maize roots. The recovery of larvae from the field grasses tested was only 12.1 ± 22.1% of that for the maize control (78.7 ± 13.6%).

Tab. 1 Percentage (compared to the maize control within each experiment) of recovery (R), dry weight (DW) and head capsule width (HC) of *D. virgifera virgifera* larvae reared on field grasses ([1] LfL – Bavarian State Research Center for Agriculture, Freising, Germany; S – seeds).

Tab. 1 *Prozentualer Anteil (im Vergleich zur Maiskontrolle in jedem Versuch) an Wiederfunden (R), Trockengewicht (DW) und Kopfkapselbreite (HC) von* D. virgifera virgifera *Larven, die an Ackergräsern gehalten wurden ([1] LfL – Bayerische Landesanstalt für Landwirtschaft, Freising, Deutschland; S – Samen).*

Taxon	Variety	Source[1]		R	DW	HC
Alopecurus pratensis	Alko	LfL	S	0.0		
Arrhenatherum elatius	Arone	LfL	S	0.0		
*Bromus mollis**	wild population	LfL	S	0.0		
*Dactylis glomerata**	Husar	LfL	S	59.3	33.9	81.5
Festuca arundinacea	Lipalma	LfL	S	2.4	11.8	61.3
F. pratensis	Cosmolit	LfL	S	29.8	31.8	69.6
F. rubra	Condor	LfL	S	0.0	0.0	0.0
Lolium multiflorum	Fastyl	LfL	S	8.3	15.4	64.2
L. multiflorum	Liberta	LfL	S	11.9	17.6	66.4
L. perenne	Ivana	LfL	S	0.0		
L. perenne	Niata	LfL	S	5.7	31.2	71.7
L. perenne	Pionero	LfL	S	15.7	20.1	65.9
L. perenne	Pomerol	LfL	S	1.4	26.0	93.2
Phalaris arundinacea	wild population	LfL	S	36.5	13.0	64.7
Phleum pratense	Phlewiola	LfL	S	9.7	15.7	63.0
Poa pratensis	Nixe	LfL	S	0.0		

* several times tested

Tab. 2 Percentage (compared to the maize control within each experiment) of recovery (R), dry weight (DW) and head capsule width (HC) of *D. virgifera virgifera* larvae reared on *Panicum virgatum* ([1] NRS – Natur-Rostoff-Service, Kanzem, Germany; S – seeds).

Tab. 2 *Prozentualer Anteil (im Vergleich zur Maiskontrolle in jedem Versuch) an Wiederfunden (R), Trockengewicht (DW) und Kopfkapselbreite (HC) von* D. virgifera virgifera *Larven die an* Panicum virgatum *gehalten wurden ([1] NRS – Natur-Rostoff-Service, Kanzem, Deutschland; S – Samen).*

Taxon	Variety	Source[1]		R	DW	HC
Panicum virgatum	Alamo	NRS	S	6.1	10.2	59.1
P. virgatum	Carthage	NRS	S	7.3	6.4	60.0
P. virgatum	Cave in Rock	NRS	S	0.0		
P. virgatum	Forestburg	NRS	S	7.3	13.8	71.4
P. virgatum	Kanlow	NRS	S	15.9	10.3	63.0
P. virgatum	Sunburst	NRS	S	1.2	4.6	66.3

Also the head capsule width of larvae feeding on field grasses was smaller than for the maize control. The largest head capsule width was recorded for larvae that developed on the forage grass var. Husar (432.7 ± 79.8 µm; n=140) and both ryegrass varieties Niata and Cosmolit (371.2 ± 65.9 µm

and 351.2 ± 60.5 µm). The head capsule width of larvae in the corresponding maize control was 521.8 ± 37.3 µm. In comparison to the larvae on maize those on ryegrasses var. Pomerol stand out, as at 93.2% they are only slightly smaller than those on maize. But as there was only a single larva extracted from this plant its food quality will be rated very low. The reduced head capsule width of larvae that developed on field grasses can be accounted for in terms of their slow development. While on maize roots most larvae (96.4%) reached the third larval stage (L3), only 38.6% reached this stage on field grasses with 60.6% still only at the second stage (L2) and a smaller percentage (0.8%) at the first stage.

The roots of most (i.e. 15) of the 18 *Sorghum* species and varieties tested were unsuitable for the development of WCR larvae. For the remaining three species of *Sorghum* a maximum of only two larvae (of 100 inoculated) were recovered (Tab. 3). These species are not seen as alternative biofuel plants to maize. Only one larva was extracted from the roots of *S. caffrorum* and *S. dochna* and two from *S. durra*. Compared with maize the other characters used for rating host quality were also strongly reduced (Tab. 3). This supports the results of other authors that analyzed a smaller number of species/varieties (e.g. Branson *et al*., 1969; Clark and Hibbard, 2004; Oyediran *et al*., 2004). Due to the low number of recoveries the results are not presented graphically.

Tab. 3 Percentage (compared to the maize control within each experiment) of recovery (R), dry weight (DW) and head capsule width (HC) of *D. virgifera virgifera* larvae reared on *Sorghum* ([1] TFZ – Technologie und Förderzentrum, Straubing, Germany; KWS – KWS Saat AG, Einbeck, Germany; JKI – Julius Kühn-Institut, Braunschweig, Germany; S – seeds).

Tab. 3 *Prozentualer Anteil (im Vergleich zur Maiskontrolle in jedem Versuch) an Wiederfunden (R), Trockengewicht (DW) und Kopfkapselbreite (HC) von* D. virgifera virgifera *Larven, die an* Sorghum *gehalten wurden ([1] TFZ – Technologie und Förderzentrum, Straubing, Deutschland; KWS – KWS Saat AG, Einbeck, Deutschland; JKI – Julius Kühn-Institut, Braunschweig, Deutschland; S – Samen).*

Taxon	Variety	Source[1]		R	DW	HC
S. bicolor	Arlys	TFZ	S	0.0		
	Biomass 150	TFZ	S	0.0		
	Branco	KWS	S	0.0		
	Bulldozer	KWS	S	0.0		
	Goliath	TFZ	S	0.0		
	Maja	TFZ	S	0.0		
	Sucrosorgo 405	TFZ	S	0.0		
	Zerberus	TFZ	S	0.0		
	wild population	JKI	S	0.0		
S. bicolor x sudanense	Green Grazer	TFZ	S	0.0		
	Lussi	TFZ	S	0.0		
	Inka	TFZ	S	0.0		
S. caffrorum	wild population	JKI	S	1.1	27.3	66.8
S. dochna	wild population	JKI	S	1.1	16.7	68.0
S. durra	wild population	JKI	S	2.2	55.7	83.5
S. nervosum	wild population	JKI	S	0.0		
S. saccaracum	wild population	JKI	S	0.0		
S. sudanense	Akklimat	TFZ	S	0.0		

These results indicate that species of *Sorghum* are very poor quality hosts for WCR as previously reported in other studies. The opposite was true for the *Miscanthus* species tested. The number of larvae recovered from *Miscanthus* x *giganteus* roots, their dry weight and head capsule widths were the same as those recorded for larvae reared on the maize control (Tab. 4). The other *Miscanthus* genotypes were less suitable than *Miscanthus* x *giganteus*, but still more acceptable than all the forage and switch grasses tested in this study. In relation to the number of larvae inoculated at the start of the experiments the number extracted varied between 14.0 ± 12.6% for *M. sacchariflorus* (Robustus) and 70.0 ± 13.3% for *M.* x *giganteus* (rhizome-plants). In comparison with the maize control this indicates 17.7% for *M. sacchariflorus* (Robustus) and 95.7% for *M.* x *giganteus* (Tab. 4). Omitting the results for the genotype Robustus the recovery of larvae from the *Miscanthus* tested was 52.3 ± 13.5%. This value is equivalent to 74.7% of larvae extracted from maize controls.

Tab. 4 Percentage (compared to the maize control within each experiment) of recovery (R), dry weight (DW) and head capsule width (HC) of *D. virgifera virgifera* larvae reared on *Miscanthus* ([1] IVT – *in-vitro*-tec Gesellschaft zur Pflanzenvermehrung für den Umweltschutz mbH; Berlin, Germany; MBT – Mendel Biotechnology, Hayward, CA, USA; RH – rhizome-plants, IV – *in vitro*-plants).

Tab. 4 *Prozentualer Anteil (im Vergleich zur Maiskontrolle in jedem Versuch) an Wiederfunden (R), Trockengewicht (DW) und Kopfkapselbreite (HC) von* D. virgifera virgifera *Larven die an* Miscanthus *gehalten wurden. ([1] IVT –* in-vitro-*tec Gesellschaft zur Pflanzenvermehrung für den Umweltschutz mbH; Berlin, Deutschland; MBT – Mendel Biotechnology, Hayward, CA, USA; RH – Rhizome-Pflanzen, IV –* in vitro-*Pflanzen).*

Taxon	Variety	Source[1]		R	DW	HC
Miscanthus x giganteus	Mxg	IVT	RH, IV	95.7	146.3	100.0
M. sacchariflorus	Robustus	MBT	IV	17.7	25.3	69.1
M. sacchariflorus x sinensis	Amuri 1	MBT	IV	78.5	23.5	72.6
M. sacchariflorus x sinensis	Amuri 2	MBT	IV	53.2	32.5	82.2
M. sacchariflorus x sinensis	Nagara	MBT	IV	50.6	60.5	84.2
M. sinensis	Hybrid	MBT	IV	50.6	29.0	81.7

The dry weight of the larvae that developed on *Miscanthus* roots varied by a factor of 11.4. The larvae that developed on *M. sacchariflorus* x *sinensis,* Amuri 1 (0.243 ± 0.168 mg) and the genotype Robustus (0.261 ± 0.134 mg) were mostly very light in weight. The heaviest larvae were extracted from *in vitro*-plants of *Miscanthus* x *giganteus* (experiment 14) and were 2.126 ± 0.932 mg, which is the largest dry weight recorded during this study. In comparison to the dry weight of larvae reared on the maize control these values correspond to 23.5% for larvae reared on Amuri 1 (Tab. 4).

The comparison of the host quality of *Miscanthus* x *giganteus* revealed only small differences between *in vitro*- and rhizome-plants (Tab. 5). Although the percentage recovery and head capsule width of larvae that developed on these plants did not differ the dry weight of those reared on *in vitro*-plants was larger (Fig. 5).

Tab. 5 Percentage (compared to the maize control within each experiment) of recovery (R), dry weight (DW) and head capsule width (HC) of *D. virgifera virgifera* larvae reared on *in vitro*- and rhizome plants of *Miscanthus* x *giganteus*.

Tab. 5 *Prozentualer Anteil (im Vergleich zur Maiskontrolle in jedem Versuch) an Wiederfunden (R), Trockengewicht (DW) und Kopfkapselbreite (HC) von* D. virgifera virgifera *Larven die an* in-vitro- *und Rhizome-Pflanzen von* Miscanthus x giganteus *gehalten wurden.*

Taxon		R	DW	HC
M. x giganteus	*in vitro*-plants	80.7	103.7	97.3
M. x giganteus	rhizome-plants	84.3	68.3	96.6

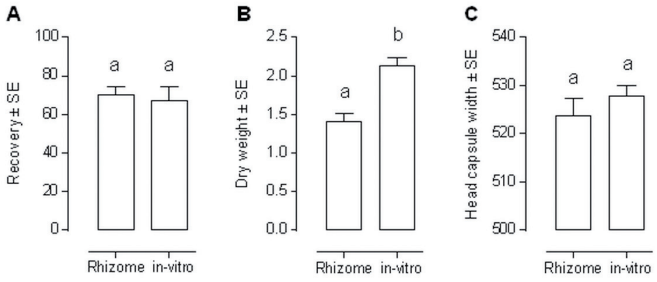

Fig. 5 Results of tests in which was analysed (A) percentage recovery, (B) dry weight (mg) and (C) head capsule width (µm) of *D. virgifera virgifera* larvae reared on *Miscanthus* x *giganteus* grown from *in vitro*- and rhizome-plants. (Different letters indicate significant differences between variants, t-test, p<0.05).

Abb. 5 *Ergebnisse der Versuche, in denen analysiert wurde (A) prozentualer Anteil an Wiederfunden, (B) Trockengewicht (mg) und (C) Kopfkapselbreite (µm) von* D. virgifera virgifera *Larven die an* Miscanthus x giganteus *gehalten wurden, welche sich aus* in vitro- *und Rhizome-Pflanzen entwickelten. (unterschiedliche Buchstaben kennzeichnen signifikante Unterschiede zwischen den Varianten, t-Test, p<0,05).*

No larvae developed on the three broad leaved species of dicotyledonous plants tested. This is in accordance with all previous studies, which used hosts other than maize (e. g. Branson and Ortman, 1967a, 1970; Behle *et al.,* 2008).

4. Conclusion

It is reported that larvae of the western corn rootworm are able to develop on plant species other than maize. Therefore, it is not surprising we recorded something similar for the biofuel plants used in this study. However, in accordance with previous studies the quality of most of these 'alternative hosts' for WCR is considerably less than that of maize, which resulted in fewer larvae becoming established and surviving and a prolonged larval development, as indicated by smaller head capsule widths and reduced dry weights. Based on the results presented the majority of the forage and switch grasses tested are not suitable for eradicating small and recently established populations. Despite this they can be used as an alternative to maize as part of a crop rotation strategy when *D. virgifera virgifera* is established in an area. The low host quality of these grasses will result in a decrease in the population size of *D. virgifera virgifera* and a diversification of the crops that can be used as biofuels.

All *Sorghum* varieties tested were not suitable hosts for WCR larvae, but are promising biofuel crops. Therefore, if grown in rotation with maize, *Sorghum* provides a highly effective and ecological friendly way of controlling WCR, and is highly productive.

That species of *Miscanthus*, especially *M.* x *giganteus,* are good hosts for WCR is alarming. The long crop cycle of *Miscanthus* rhizomes of up to 20 years would appear to provide an excellent and long lasting source of high numbers of WCR adults, as continuous maize production does. However, this depends on the egg laying behaviour of the females. Only if they lay their eggs in established *Miscanthus* stands they will complete their life cycle there. Despite some early findings of studies carried out in the US this question is still unanswered and should be evaluated at a large spatial scale. Based on what is known about the oviposition behaviour of WCR they lay very few eggs in crops other than maize. As the oviposition sites are located very close to adult feeding sites, e. g. plants that produce large amounts of pollen, it is very unlikely that WCR will lay eggs in crops of *Miscanthus*.

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References

BBCH working group, 2001: Growth stages of mono-and dicotyledonous plants. BBCH Monograph. Document available via: http://pub.jki.bund.de/index.php/BBCH/article/view/515.

Behle, R. W., B. E. Hibbard, S. C. Cermak and T. A. Isbell, 2008: Examining *Cuphea* as a potential host for western corn rootworm (Coleoptera: Chrysomelidae): Larval development. Journal of Economic Entomology **101**: 797–800.

Branson, T. F., 1976: The selection of a non-diapause strain of *Diabrotica virgifera* Coleoptera: Chrysomelidae). Entomologia Experimentalis et Applicata **19**: 148–154.

Branson, T. F., P. L. Guss, J. L. Krysan and G. R. Sutter, 1975: Corn rootworms: laboratory rearing and manipulation. USDA Agric. Res. Serv. Bull. **28**: 18 pp.

Branson, T. F., P. L. Guss and E. E. Ortman, 1969: Toxicity of sorghum roots to larvae of western corn rootworm. Journal of Economic Entomology **62**: 1375–1378.

Branson, T. F., J. J. Jackson and G. R. Sutter, 1988: Improved method for rearing *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Journal of Economic Entomology **81**: 410–414.

Branson, T. F. and E. E. Ortman, 1967a: Host range of larvae of western corn rootworm. Journal of Economic Entomology **60**: 201–203.

Branson, T. F. and E. E. Ortman, 1967b: Fertility of western corn rootworm reared as larvae on alternate hosts. Journal of Economic Entomology **60**: 595.

Branson, T. F. and E. E. Ortman, 1970: Host range of western corn rootworm: further studies. Journal of Economic Entomology **63**: 800–803.

Breitenbach, S., U. Heimbach and K. Lauer, 2005: Field tests on the host range of the larvae of the Western corn rootworm (*Diabrotica virgifera virgifera* LeConte 1868, Chrysomelidae, Coleoptera). Nachrichtenblatt des Deutschen Pflanzenschutzdienstes **57**: 241–244.

Cabrera Walsh, G., 2007: *Sorghum halepense* (L.) Persoon (Poaceae), a new larval host for the South American corn rootworm *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae). Coleopterists Bulletin **61**: 83–84.

Chege, P. G., T. L. Clark and B. E. Hibbard, 2005: Alternate host phenology affects survivorship, growth, and development of western corn rootworm (Coleoptera: Chrysomelidae) larvae. Environmental Entomology **34**: 1441–1447.

Clark, T. L. and B. E. Hibbard, 2004: Comparison of nonmaize hosts to support western corn rootworm (Coleoptera: Chrysomelidae) larval biology. Environmental Entomology **33**: 681–689.

Hibbard, B. E., Y. M. Schweikert, M. L. Higdon and Ellersieck, 2008: Maize phenology affects establishment, damage, and development of the western corn rootworm (Coleoptera: Chrysomelidae). Environmental Entomology **37**: 1558–1564.

MacFadyen, A., 1961: Improved funnel-type extractors for soil arthropods. Journal of Animal Ecology **30**: 171–184.

Moeser, J. 2003: Nutritional ecology of the invasive maize pest *Diabrotica virgifera* LeConte in Europe. PhD thesis, Göttingen.

Moeser, J. and S. Vidal, 2004: Do alternative host plants enhance the invasion of the maize pest *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae, Galerucinae) in Europe? Environmental Entomology **33**: 1169–1177.

Oyediran, I. O., B. E. Hibbard and T. L. Clark 2004: Prairie grasses as hosts of the Western corn rootworm (Coleoptera: Chrysomelidae). Environmental Entomology **33**: 740–747.

Spencer, J. L. and S. Raghu, 2009: Refuge or Reservoir? The Potential Impacts of the Biofuel Crop *Miscanthus* x *giganteus* on a Major Pest of Maize. PLoS ONE 4.

Wilson, T. A. and B. E. Hibbard, 2004: Host suitability of nonmaize agroecosystem grasses for the western corn rootworm (Coleoptera: Chrysomelidae). Environmental Entomology **33**: 1102–1108.