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In both sites there is a significant linear regression between the years since the commodity started to function and the level of infestation. This can suggest that the facility itself, during the course of the years, accumulates increased amount of insects and treatment in-between storage is not sufficient or the insects developed resistance to pesticides (e.g. previous studies [10]). We are currently studying populations' dynamics within the year to reveal if a new harvest of grains initiates with high infection or if the infection level is equal in all years at the beginning of the storages but reacts differently to insecticides.

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Innovative stored plant products in Germany and the potential threat by native and invasive pest insects

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Abstract

Climate change, economic-political developments as well as new trends in diet and in bio-economy considerably influence the assortment of cultivated plants in Germany and thereby, determine the plant products which have to be stored after harvest. In the light of the International Year of Pulses 2016 and also, as a result of the European Soya Declaration, the acreage cultivated with new plants such as pulses, stress tolerant wheat varieties and also oil seed rape expanded worldwide. Due to increasing stocks of novel commodities, the emergence of economically important insects infesting stored products and the possible risk caused by native and invasive pest species have to be generally considered during storage. In this overall context, we studied the capacity of various stored-product pest insects to infest two important pulses. In laboratory tests different varieties of soy and lupine have been offered as whole seeds, grist and flour to selected moth and beetle species common in Germany. Over 14 weeks we examined the developmental time from egg to eclosion as well as the number of adults in the F1 generation compared to control insects reared on their standard feeding substrate. First findings under laboratory conditions (20-25 °C, 65-70 % RH) indicate that these innovative stored products, and in particular its simply processed plant products are highly susceptible to moths (i.a. Ephestia elutella, Plodia interpunctella) and to a much lesser extent also to some beetle species (i.a. Callosobruchus chinensis, Tribolium *confusum*), but the usally recommended optimal storage conditions (T \leq 16 °C, RH \leq 65%) can prevent a loss of volume and quality.

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Keywords: pulses, soy, lupine, stored-product pests, risk of infestation

Introduction

Following the goals of the United Nations Agenda 2030 adopted in 2015 (United Nations, 2016) and the European Union's Sustainable Development Strategy adopted in 2001 (Commission of the European Communities, 2001), the German Federal Ministry of Food and Agriculture (BMEL) has started to prioritise food security and improve nutrition. In addition, the BMEL has stepped up his support for more sustainable and resilient agricultural systems, responding to advancing climate change, social-political developments and new consumer trends in diet and bio-economy.

As part of the German engagement, promoting the production of legume crops destinated for animal and human consumption, a so-called "protein crop strategy" has been initiated to improve cultivation of field beans and peas as well as that of more alternative protein consisting crops such as soy bean and various lupine species ("Eiweißpflanzenstrategie", BLE announcement, No. 20/17/31, 2017). Moreover, in July 2017 fourteen European ministers have signed the European Soy Declaration (Council of the European Union, 2017), an initiative launched by the German and the Hungarian Minister of Agriculture. Both political strategies underline that legume crops are vital to the agricultural system in Europe. As only 3% of the arable land in Europe is used for legume crops, the political guidelines aim to encourage the cultivation of pulses on the one hand and the scientific research related to the primary production of protein plants on the other. In this way, the participating countries will gain greater independence from non-European food and feed imports and also, support a diversification of crop rotation.

The domestic lupine (*Lupinus* spp.) and soybean (*Glycine max*), which have been introduced to Europa 150 years ago, represent excellent alternative protein sources and a protein-rich feedstuff. Both pulses have a protein content of up to 45% and thus, provide valuable plant-based protein for the production of food, feed and nutritional supplements (Bader et al., 2009; Hartman et al., 2011).

The yields of these legume crops per ha in Europe are comparatively high (e.g. to date in Germany the yield of soy is 34.4 dt/ha and the yield of lupine 18.2 dt/ha; Federal Statistical Office, 2017) and for soybean similar to those recorded in the USA and Brazil (www.usda.com; www.soystats.com). Nevertheless, Germany has a protein gap of approx. 2.5 million tons, which represents 65% of the 3.66 million tons of protein consumed in 2015 and therefore, still needs to import ca. 3.5 million tons of soy per year (http://www.fao.org/faostat/en/#home; www.ovid-verband.de). For this reason, it is a first success of the European and national politics that the area of soybean, cultivated mainly in southern parts of Germany, has been increased from estimated 5,000 ha in 2011 to 19,000 ha in 2017 (Donausoja, 2017). The harvested amounts for their part have increased from 13,000 to 66,000 t which represents a fourfold increase of the amount of inland harvested soybeans (Federal Statistical Office, 2017).

Currently, three agricultural lupine species are cultivated in Germany, the blue lupine (*Lupinus angustifolius*), the yellow lupine (*L. luteus*) and the white lupine (*L. albus*) (Bader et al., 2009; Bremer, 1999). The blue sweet lupine species, for example, are cultivated on about 30,000 hectares in the North German region (Ruge-Wehling et al., 2016) and about 53,000 t were harvested in 2017 (Federal Statistical Office, 2017).

In total, the arable acreage of pulses in Germany reached nearly 197,000 ha in 2017 (Federal Statistical Office, 2017). With increasing yields the stored amounts of these pulses will grow as well and aspects of stored-product protection have to take into consideration. Nevertheless, in case of infestation some main pesticides could be used for chemical protection of pulses, oilseeds and expeller but no pesticide especially approved for the use in stored soy and lupine is available in Germany.

Moreover, very little is known about stored-product pests associated with soy and/or lupine and their potential to develop on these plant products and consequently the damage they could provoke in middle latitudes. More than 60 insect species are described to occur in soy storages

(Hagstrum and Subramanyam, 2009) but central European storekeepers state that up to date pest insects do not represent an economic risk during storage of soy. Only the Mediterranean flour moth has been observed in greater numbers during storage in big bags (Ghosh and Jayas, 2010).

To get recommendations for good storage practice, the present study should clarify the following questions:

- 1) Can local stored-product pests infest stored soy and lupine and develop successfully?
- 2) How do they develop compared to those reared on standard (control) feeding substrate?
- 3) Does the processing level/degree of plant products have any influence on the development of pest insects?
- 4) How is the respective damage pattern provoked by the tested pest species?

Materials and Methods

To study the potential of common (native and invasive) stored-product pests to develop on soy and lupine adults or eggs of the respective beetle and moth species were placed together with 200 g of whole beans as well as of the simply processed plant products (grist and flour) of these pulses in rearing glass jars (3 L) covered with cotton cloth (Tab. 1). For experiments with soy the differently processed substrates were infested by adding 10 adult moths or 30 adult beetles. Whole beans of lupine were infested by adding 100 moth eggs or 50 adult beetles. Test beetles were all of the same age without taking into account sexes. Additionally, grist and flour of a mix of 4 blue sweet varieties (*Boregine, Boruta, Mitrabor, Probor*), one white sweet variety (*Energy*) and one blue bitter variety (*Karo ZS*) were infested by adding 100 moth eggs of *Plodia interpunctella* and *Ephestia elutella* at the beginning of the experiment. As control the indicated amounts of the respective standard laboratory feeding substrates (equivalent to the test substrates) for each pest species were used (Tab. 1). Each treatment was replicated 6 times.

After one week the beforehand added adults were removed and glass jars were maintained at 22 or 25 ± 1 °C and 65-70 % RH in climate chambers (Tab. 1). The glass jars were checked every two days and newly hatched adults were removed. We measured the time until first hatchings of new (F1) adults and counted the number of fully developed individuals. The mean development time from egg to adult and the number of hatched adults on soy and lupine were compared with those reared on control feeding substrate (Tab. 1). Experiments were stopped when in control jars no further hatching was observed during 5 days.

Results & Discussion

In the present study we showed that the stored-product moth species *E. elutella* and *P. interpunctella* have the potential to multiply on stored pulses, especially on its simply processed forms, grist and flour (Tab. 2 - 4). Progeny of both moth species tested here developed well on soy beans and the corresponding processed substrates (Tab. 2). Mean number of hatched adult *P. interpunctella* on soy grist and flour was comparable to those on the control standard feeding substrate. But the development time was significantly longer on soy and hatching start of F1 adults was shifted for 3 weeks in *E. elutella* and for 2 weeks in *P. interpunctella* (Tab. 2).

Moreover, *P. interpunctella* showed the potential to develop on lupine beans and new F1 adults hatched on all varieties tested but also, significantly later and to a much lesser extent than on the control feeding substrate (Tab. 3). Most adults were counted on the white sweet lupine variety *Energy* followed by the blue bitter one *Karo ZS* and the blue sweet variety *Boruta*. On the processed plant material of lupine, *P. interpunctella* and *E. elutella*, developed better than on whole beans and in comparable numbers to the standard feeding substrate but hatching start was shifted for 2-3 weeks as well (Tab. 4). Highest numbers of hatched larvae of *P. interpunctella* were found on grist and flour of the sweet lupine mix (100% compared to control). In the two experiments, live larvae and adult individuals of moths were found and damage was displayed by feeding traces, feces and webs (Tab. 4). However, under laboratory conditions the tested stored-product pest beetles did not

develop or not at all on the different substrates and thus, demonstrated a very low risk of infestation. This is possibly due to the biology of these species which are most probably specialized on other feeding substrates than soy and lupine. Even bruchids that might start to colonize these seeds in the field directly on plant could not develop properly. In this context, no live adults of *Sitophilus granarius* were found and the substrate was totally undamaged. Some *Callosobruchus chinensis* individuals developed at 22°C on soy beans and at 25°C on soy grist as well but significantly fewer adults were found compared to the control substrate. *Tribolium confusum* developed better on the processed grist and flour than on whole soy beans but hatching start of F1 adults was shifted for more than 5 weeks (Tab. 2). Observed damage patterns were live individuals and the typical smell associated with *Tribolium*-infestation. On the six varieties of sweet and bitter lupine beans none of the three tested beetle species developed and only some *Rhyzoperta dominica* adults were found (Tab. 3). The significant longer development time until hatching of new *P. interpunctella* and *T. confusum* adults on soy and flour at 25°C and 65% RH compared to the standard feeding substrate (Tab. 2) is comparable to data from literature (Cox and Simms 1978).

Tab. 1 Experimental design to test the development time from egg to adult (F1) of different stored-product pest species (moth and beetle) on 200 g of whole beans, grist or flour of one soy variety (*Sultana*) and six lupine varieties (blue sweet (*bs*): *Boregine, Boruta, Mitrabor, Probor;* white sweet (*ws*): *Energy;* blue bitter (*bb*): *Karo ZS*), as well as on the corresponding standard fedding substrates as control (N=6).

PESTS	PULSES	SOY beans, grist, flour	LUPINE beans (I-VII), grist+flour (V-VII)							
	CONTROL Atajuan	Sultana	I) Boregine (bs)	II) Boruta (bs)	III) Mirabor (bs)	IV) Probor (bs)	V) Sweet mix+ (bs)	VI) Energy (ws)	VII) Karo ZS (bb)	
Moths	Standard feeding substrate	Infested with								
Ephestia elutella	Wheat bran (200g)	10 adults	-	-	-	-	100 eggs*	100 eggs*	100 eggs*	
Plodia interpunctella	Wheat bran/almond grist (185/15g)	10 adults	100 eggs	100 eggs	100 eggs	100 eggs	100 eggs*	100 eggs*	100 eggs*	
Beetles										
Acanthocelides obtecus	Black-eyed beans (100g)	-	50 adults	50 adults	50 adults	50 adults	-	50 adults	50 adults	
Callosobruchus chinensis	Peas (200g)	30 adults		-	-	-	-	-		
Callosobruchus maculatus	Mung beans (100g)	-	50 adults	50 adults	50 adults	50 adults	-	50 adults	50 adults	
Rhyzopertha dominica	Wheat grain (200g)	-	50 adults	50 adults	50 adults	50 adults	-	50 adults	50 adults	
Sitophilus granarius	Wheat grain (200g)	30 adults	-	-	-	-	-	-		
Tribolium confusum	Wheat grist/yeast (191/9g)	30 adults	-	-	-	-	-	-		

+ Mix of 4 blue sweet lupine varieties: Boregine, Boruta, Mirabor, Probor.

* Additional experiment by infesting grist and flour of lupine sweet mix (V) and varieties VI and VII with 100 moth eggs each.

Since development not only depends on the feeding substrate but also on factors such as temperature and humidity (Dettner and Peters, 2011), the experiments presented here (under specified temperature, product moisture and relative humidity) only give a first indication of whether the tested species represent a real risk to stored soy and lupine. In fact, higher temperatures seem to favor the potential of beetles and pests of tropical origins to develop on the tested feeding substrates. Therefore, during cold, dry and well-ventilated storage these pest insects probably do not represent a high risk for the stored pulses. Here, the temperature effect has been observed on *C. chinensis* (Tab. 2) which may be an indication of the potential for increased reproduction on soy at higher temperatures. This in turn implies that the potential to develop on innovative stored products (pulses) in Germany may rise with increasing global warming.

In any case, a thorough cleaning before storage of soy and lupine is to be recommended, in order to prevent the spreading of harmful insects, even after a nonmonitored infestation. Consequently, the most important preventive measures against pest insects and future infestations in practice are

well-cleaned storage facilities, cool storage temperatures (10-16 ° C) and for long-term storage kernels with no more than 11% residual moisture (Landwirtschaftliches Zentrum für Sojaanbau und Entwicklung, 2015).

Tab. 2 The potential risk of stored soy beans, grist and flour to get infested by common stored-product pests. Summary of experiments analyzing the capability of different moth and beetle species to develop on whole beans, grist and flour of the soy variety (*Sultana*) and measuring the developmental time from egg to adult (F1) compared to standard control substrates.

PESTS ON <u>SOY</u> (Sultana)	Development time compared to control (weeks)			Mean n° of hatched adults compared to control (%)				
(Suitana)	beans	grist	flour	beans	grist	flour	Damage pattern	Risk of infestation
Moths								green levels: low risk red levels: potential risk
P. interpunctella (at 25°C)	>	>	>	22.1	75.7	80.1	Feces Webbing Larvae	High potential to infest soy, especially the processed forms, grist and flour. Loss of quality due to moth webs and larvae. Moth develop well.
E. elutella (at 25°C)	>>	>>	>>	20.2	57.1	54.8	Living individuals Feces Webbing Larvae	High potential to infest soy, especially the processed forms, grist and flour. Moth develop well. Loss of quality due to moth webs and larvae.
Beetles								
T. confusum (at 24°C)	>>>	>>>	>>>	0.3	8.2	6.3	Living individuals Typical smell	Higher risk of infestation on soy grist and flour at warmer temperatures.
C. chinensis (at 25°C)	>>	>>	x	3.1	5.0	x	Living individuals Laid eggs No drill holes	Risk of infestation on soy beans and grist increases with increasing temperatres .
C. chinensis (at 22°C)	>>	x	x	0.5	x	x	Living individuals Laid eggs No drill holes	Very little risk of infestation and only on soy beans.
S. granarius (at 20°C)	x	x	x	x	x	x	None	No expected infestation since beans are toobig and without the necessary endosperm.

>: Development time slightly longer than on control substrate (shift ca. 2 weeks)

>>: Development time longer than on control substrate (shift ca. 3 weeks)

>>>: Development time much longer than on control substrate (shift > 5 weeks)

X: No development (no adult individuals hatched)

Tab.3 The potential risk of stored lupine beans to get infested by common stored-product pests. Summary of experiments analyzing the capability of different moth and beetle species to develop on whole beans of six lupine varieties (*Boregine, Boruta, Energy, Mitrabor, Probor, Karo ZS*) and measuring the developmental time from egg to adult (F1) compared to standard control substrates.

PESTS ON LUPINE		t time compared tched adults co						
whole beans	Boregine (bs)	Boruta (bs)	Mirabor (bs)	Probor (bs)	Energy (ws)	Karo ZS (bb)	Damage pattern	Risk of infestation
Moths								green levels: low risk red levels: potential risk
P. interpunctella (at 25°C)	>>/ 3.0	>>/ 5.1	>>/ 2.5	>>/ 3.8	>>/ 8.5	>>/ 5.0	Living individuals Feces Webbing Larvae	Some potential to infest all varieties of sweet and bitter lupine whole beans. Loss of quality due to moth webs and larvae.
Beetles								
R. dominica (at 25°C)	>>>/ 0.2	>>>/ 0.4	>>>/ 0.2	>>>/ 0.3	>>>/ 0.4	>>>/ 0.1	Verylow	Almost no risk of infestation on all varieties of lupine whole beans.
C. maculatus (at 25°C)	x	x	x	x	x	x	None	No expected infestation.
A. obtecus (at 25°C)	x	x	x	x	x	x	None	No expected infestation.

>>: Development time longer than on control substrate (shift ca. 3 weeks)

>>>: Development time much longer than on control substrate (shift > 5 weeks)

X: No development (no adult individuals hatched)

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Tab.4 The potential risk of lupine grist and flour to get infested by common stored-product pests. Summary of experiments analyzing the capability of *P. interpunctella* and *E. elutella* (100 eggs initially) to develop on grist and flour of a mix of 4 blue sweet varieties (*Boregine, Boruta, Mitrabor, Probor*), one white sweet variety (*Energy*) and one blue bitter variety (*Karo ZS*) and measuring the developmental time from egg to adult (F1) compared to standard control substrates.

	Development time compared to control (weeks) / Mean n° of hatched adults compared to control (%)								
PESTS ON <u>LUPINE</u> grist and flour	Sweet mix (bs)		Energy (ws)		Karo ZS (bb)		Damage pattern	Risk of infestation	
	grist	flour	grist	flour	grist	flour		green levels: low risk red levels: potential risk	
P. interpunctella (at 25°C)	>/ 100	>/ 100	>/ 89	>/ 76	>/ 98	>/ 97	Living individuals Feces Webbing Larvae	High potential to infest processed lupine (grist and flour). Moth develop well. Loss of quality due to moth webs and larvae.	
E. elutella (at 25°C)	>/ 83	> / 92	>/ 90	>/ 81	>/ 98	>/ 97	Living individuals Feces Webbing Larvae	High potential to infest processed lupine (grist and flour). Moth develop well. Loss of quality due to moth webs and larvae.	

>: Development time slightly longer than on control substrate (shift ca. 2 weeks)

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Biological abilities of storage pests required for the successful penetration of food packages or seeds

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