

## Investigations into the ecology of the *Wheat dwarf virus* (WDV) in Saxony-Anhalt, Germany

### Untersuchungen zur Ökologie des *Wheat dwarf virus* (WDV) in Sachsen-Anhalt, Deutschland

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#### Summary

The *Wheat dwarf virus* (WDV) is widespread in Saxony-Anhalt, Germany. The barley strain was found in barley, oats, maize and in different species of grasses, the wheat strain in wheat. In triticale, both strains were detected. While in the past WDV incidence increased mainly in winter barley, it now attacks also early sown winter wheat and triticale. Self-sown cereals and grasses serve as infection sources. *Bromus arvensis* L., *B. commutatus* Schrader, *B. hordeaceus* L., *B. japonicus* Thunb. ex Murray, *B. sterilis* L. and *Phalaris arundinacea* L. have been identified as new host plants of WDV. Individuals of *Psammotettix alienus* Dahlb. are able to transmit WDV in all stages of their development, but the transmission mode is subject to very broad fluctuations. Virus acquisition by individuals reached 50.0 % in the L2-stage, 45.0 % in L3-stage, 42.9 % in L1-stage, 40.5 % in the imago-stage, 23.6 % in L4-stage and 9.1 % in the L5-stage. The percentage of viruliferous *P. alienus* in a field population varied in dependence on the time of year (June: about 80.0 %; July to October: about 10.0–25.0 %; November to December: about 40.0 %). In single cases, a leafhopper may host both strains of WDV.

**Key words:** *Wheat dwarf virus* (WDV); *Psammotettix alienus* Dahlb.; host range; virus transmission; strain spectrum

#### Zusammenfassung

Das WDV ist in Sachsen-Anhalt, Deutschland weit verbreitet. In Gerste, Hafer, Mais und verschiedenen Grasarten kommt der Gerstenstamm des WDV vor, in Weizen hingegen der Weizenstamm. In Triticale wurden beide Stämme gefunden. Während bisher das WDV besonders in Wintergerste zunehmend auftrat, werden neuerdings auch früh gedrillter Winterweizen und Triticale befallen. Als Infektionsquellen sind Ausfallgetreide und Wildgräser zu nennen. Als neue Wirtspflanzen wurden gefunden: *Bromus arvensis* L., *B. commutatus* Schrader, *B. hordeaceus* L., *B. japonicus* Thunb. ex Murray, *B. sterilis* L. and *Phalaris arundinacea* L. Alle Entwicklungsstadien von *Psammotettix alienus* Dahlb. sind befähigt, das WDV zu übertragen, wobei der individuelle Übertragungsmodus große Unterschiede aufweist. Bei einer Virusaufnahme im zweiten Larvenstadium (L2) lag die Effektivität der Übertragung bei 50,0 %. Das L3-Stadium erreichte 45,0 %, L1 42,9 %, Imagines 40,5 %, L4 23,6 % und das L5-Stadium 9,1 %. Der Prozentsatz virustragender *P. alienus* in der Feldpopulation schwankte

in Abhängigkeit von der Jahreszeit (Juni: ca. 80,0 %; Juli bis Oktober: ca. 10,0–25,0 %; November bis Dezember: ca. 40,0 %). In Einzelfällen können die Zikaden beide Stämme des WDV übertragen.

**Stichwörter:** Weizenverzweigungs-Virus (WDV); *Psammatettix alienus* Dahlb.; Wirkkreis; Virusübertragung; Stammspectrum

## 1 Introduction

*Wheat dwarf virus* (WDV) is rather widespread in Saxony-Anhalt (Germany). In this region, the virus infects barley, wheat, rye, oats and triticale as well as different species of wild grasses. In the last years, WDV appeared in cereal crops more often than *Barley yellow dwarf viruses* (BYDVs) and *Cereal yellow dwarf virus* (CYDV) (in the following BYDVs). A lot of important questions concerning WDV are still open. Therefore, intensive investigations into the ecology of the virus and its vector *Psammatettix alienus* Dahlb. have been carried out since 1995. The examinations focused on various issues: Surveying WDV incidence in self-sown and in new-sown cereal fields, investigations of the host range of the virus, identification of the strain spectrum of WDV in Saxony-Anhalt and determination of WDV transmission by the vector *P. alienus*.

WDV appears in two different strains, preferring barley or wheat as host plants. Some host plants are known for the presence of both strains (LINDSTEN and VACKE 1991).

## 2 Material and methods

### 2.1 Investigation of WDV incidence

Since 1995, 20 to 30 sites with a lot of shatter grain have been checked each year for WDV and BYDVs using DAS-ELISA. In each field, 20 symptomatic single plants were checked on average. This survey was to reveal the relative frequency of the viruses.

Adjacent to severely WDV infested fields with shatter grain, new sowings were tested for WDV and BYDVs. The area was sampled in autumn (November to December) and in spring (March to April, prior to the hatching of *P. alienus*); selected locations also in early summer (end of June). Thirty subsequent single plants from four sides of the field each and the centre served as test material. In autumn 2000, winter wheat and triticale fields were checked for the first time.

The DAS-ELISA tests followed mainly the description given by FLEGG and CLARK (1979).

### 2.2 Examination of the host range of WDV

In 1999 and 2000, altogether 54 grass species were examined for their aptitude as WDV host. They included grasses that had earlier been described as natural host plants of WDV (VACKE 1972, VACKE and CIBULKA 1999) and other selected grass genera and species frequently found in Saxony-Anhalt (BENKERT et al. 1996). Each year, the plants were grown in the greenhouse in March, and in mid-May 10 to 15 single plants per species were planted in fields of winter barley strongly infested by WDV. Thus, the plants were exposed to high natural infection pressure. In mid-July, all single plants were tested for WDV and BYDVs by use of ELISA. All WDV-infested plants were checked for the occurrence of both virus strains by means of PCR. Likewise, maize was checked for its host suitability. Within 3 years (1997, 1998, 2000), altogether four cultivars as well as 17 inbred lines and single cross hybrids were undersown in infected barley fields and serologically tested in July.

### 2.3 Identification of WDV strain spectrum in Saxony-Anhalt

From each WDV-infected field, if available, 20 infected single plants were collected and, by use of PCR, tested for the occurrence of the barley or wheat strain. The virus DNA was extracted according to PALMER et al. (1998). Position and orientation of used primers (universal primers P1 and P2, strain-specific primers P3 and P4) were in agreement with the information given by COMMANDEUR and HUTH (1999).

## 2.4 Effectiveness of transmission of *Psammatettix alienus* Dahlb.

The only WDV vector known so far is the leafhopper *P. alienus* (VACKER 1961). After the introduction of rearing (MANURUNG et al. 2001), first experiments were started into the interrelationship between virus and vector. As a first step, the different development stages of *P. alienus* (L1 to L5 and imagines) were tested for the effectiveness of transmitting WDV. Virus-free leafhoppers of the corresponding development stages were put on a WDV-infected barley plant for 24 h (isolate from the site Zscherben); subsequently, the animals were individually conveyed to barley plants (cv. 'Theresa'/2 to 3-leaf stage). When the next stage of development was reached and in case of adult leafhoppers, a further transmission to a healthy plant was carried out 5 days later. The inoculated plants were brought to a greenhouse and tested by means of DAS-ELISA 4 weeks p. i. The transmission experiments were made under laboratory conditions: 20 °C, 70–95 % relative air humidity, 18 h lighting and 6 h darkness.

## 2.5 Detection of viruliferous *P. alienus* in barley fields

In intervals of 2 weeks between June and December 2000, individuals of *P. alienus* were caught by means of an insect-net in a crop of WDV-infected winter barley in Zscherben. Immediately afterwards, about 25 individuals were separately set on healthy plants of winter barley. Eight days later, the leafhoppers were transmitted to plants of winter wheat, again one specimen per plant for a feeding period of 5 days. Four weeks p. i., all single plants were serologically retested. The leafhoppers were preserved in alcohol to allow checkups of the share of viruliferous animals in other studies and also the occurrence of WDV strains in single leafhoppers.

# 3 Results

## 3.1 Investigations of WDV incidence

In self-sown cereal fields, WDV was more widespread than BYDVs in most years (Fig. 1). Only in 1995, BYDVs were dominating. This proportion was also evident in the virus incidence in autumn-sown cereal crops (Fig. 1). Above this, it was realized that in the new sowings of winter barley WDV infestation had increased in the last years. In autumn 2000, a total of 11 winter barley fields were surveyed. The mean WDV infestation was 13.5 %, the mean BYDVs rate 5.8 %. There was no clear relationship to the sowing date. In spring 2001, 9.6 % of the plants were infected by WDV, and 40.1 % by BYDVs. In autumn 2000, extended WDV-infected winter wheat and triticale crops were recorded for the first time (Table 1). In five surveyed fields, the autumn infection by WDV or BYDVs was 7.4 % and 4.4 %, resp. In spring 2001, the corresponding infection rates reached 9.6 % (WDV) and 40.1 % (BYDVs), resp. Wheat that had been sown at the beginning of November (site Langenbogen) remained free of viruses. Similar infection rates were found for triticale (Table 1). Averaging the four sampled fields, the autumn infection reached 1.7 % (WDV) and 6.8 % (BYDVs). The corresponding levels in spring were 0.7 % (WDV) and 47.2 % (BYDVs), resp.

As a result of the considerable appearance of aphids in autumn 2000 and their anholocyclical overwintering, an epidemic expansion of BYDVs was observed in winter barley and early-sown crops of wheat and triticale (Table 1). Single crops showed infection rates of more than 80.0 %. In order to determine the degree of spring infection, selected fields of winter barley were again tested in the early summer (early June) of the growth periods 1998/99 and 1999/00. While new infections by WDV reached 6.3 % in 1998/99 and 17.8 % in 1999/00, they were minor in case of BYDVs in both years (0.3 %) (Table 2).

## 3.2 Examination of the host range of WDV

The studies of the host range of WDV confirmed some of the known grass species as host plants, for example *Avena fatua* L., *A. sterilis* L., *Bromus inermis* Leyss., *B. secalinus* L., *B. tectorum* L., *Lagurus ovatus* L., *Lolium remotum* Schrk., *L. temulentum* L., *Poa annua* L. As new, naturally susceptible species were identified: *Bromus arvensis* L., *B. commutatus* Schrad., *B. hordeaceus* L., *B. japonicus* Thunb. ex

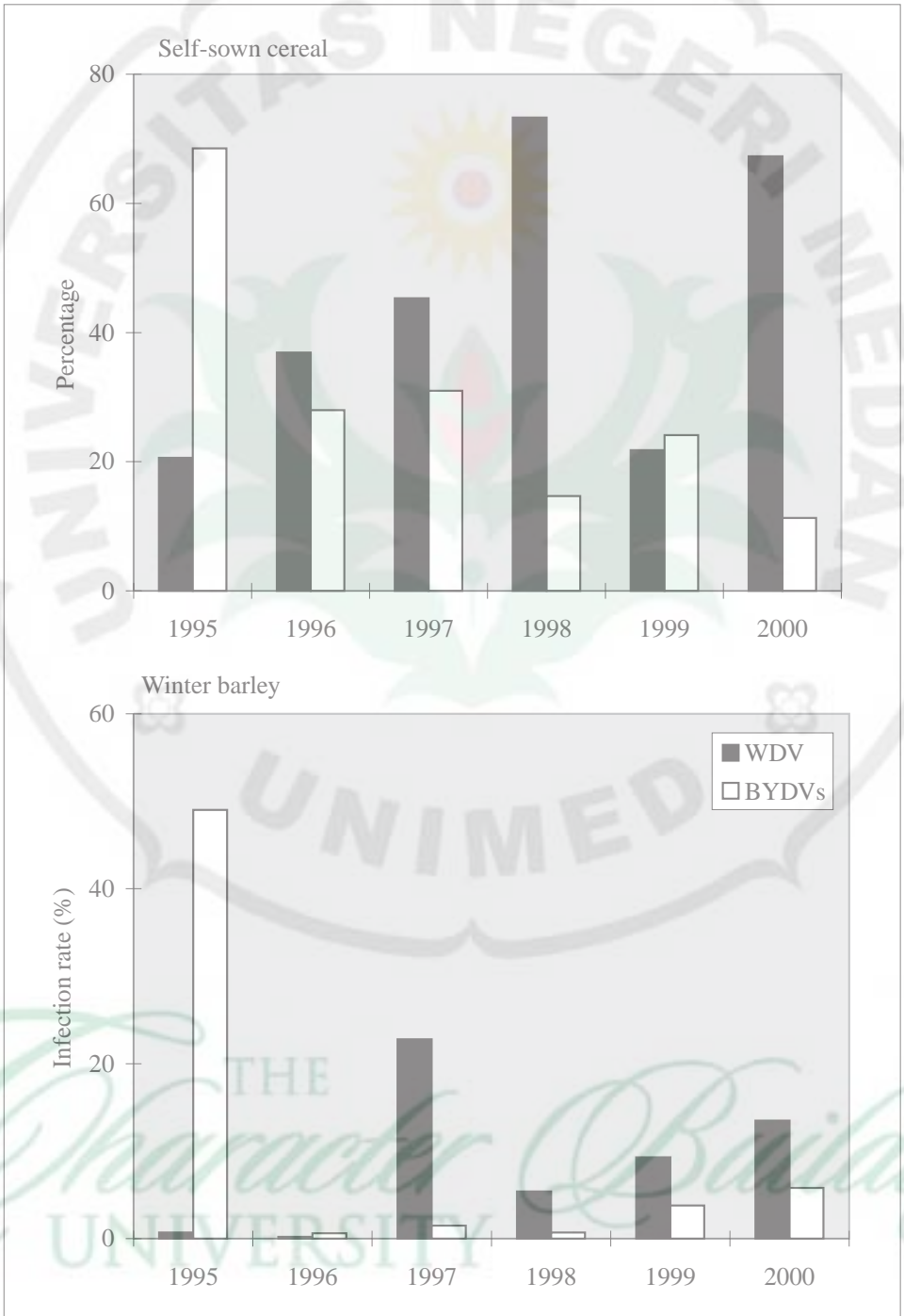


Fig. 1. Incidence of WDV and BYDVs in self-sown cereal and in winter barley in the autumn from 1995 to 2000.



Table 1. Incidence of WDV and BYDVs in barley, wheat, and triticale in autumn 2000 and in spring 2001 in Saxony-Anhalt

Fields	Sowing Date	Infection rate (%)			
		Autumn 2000		Spring 2001	
		WDV	BYDVs	WDV	BYDVs
<b>Barley</b>					
Adendorf	28.08.00	5.3	10.0	5.3	10.7
Spickendorf	11.09.00	32.7	9.3	18.7	40.0
Freist	17.09.00	22.7	8.7	5.3	66.0
Höhnstedt I	15.–21.09.00	14.0	1.3	18.0	6.0
Zappendorf	20.–24.09.00	8.0	10.0	3.3	69.3
Friedeburg	21.09.00	20.0	2.0	16.0	0.0
Wils	21.09.00	0.7	0.0	13.3	2.7
Zaschwitz	24.–27.09.00	10.0	4.7	6.7	50.7
Höhnstedt II	25.09.00	10.7	0.0	4.7	26.7
Rumpin	28.09.00	9.3	8.0	6.0	22.7
Average		13.5	5.8	9.8	30.4
<b>Wheat</b>					
Zappendorf	08.–10.09.00	9.3	3.3	5.3	48.0
Bennstedt	10.–11.09.00	10.7	21.3	30.7	66.0
Langenbogen I	11.09.00	6.7	1.3	9.3	54.0
Spickendorf	11.09.00	8.4	1.6	2.7	32.7
Langenbogen II	05.–07.11.00	0.0	0.0	0.0	0.0
Average		7.4	4.4	9.6	40.1
<b>Triticale</b>					
Benkendorf	06.09.00	1.3	25.3	1.3	56.0
Köllme	06.09.00	2.7	2.0	1.3	66.7
Zappendorf	08.09.00	0.7	0.0	0.0	62.0
Höhnstedt II	27.09.00	2.0	0.0	0.0	4.0
Average		1.7	6.8	0.7	47.2

Murray, *B. sterilis* L. and *Phalaris arundinacea* L. Most tested grass species developed no symptoms. Only some *Bromus* species responded to WDV infection by retarded growth and clear reddening. Plants of the genus *Lolium* which had been attacked by WDV developed streaked leaves. Of altogether four examined maize cultivars and five inbred lines, only the line FAP 1360 A showed susceptibility to WDV under natural conditions.

In the different years, infection rates reached 23.9 % (1997), 6.8 % (1998) and 12.5 % (2000) which implies a susceptibility that is comparable to other cereal crops.

In 1998 and 2000, 12 single cross hybrids with the line FAP 1360 A were undersown in a WDV-infected winter barley field. Serological tests made in July helped to identify infected plants of different single cross hybrids, which allow to suggest a dominant inheritance. Additionally, in 1997 about 1,400 single plants of various maize cultivars were serologically tested for WDV infection on different locations. Evidence of virus presence was not obtained in any case.

### 3.3 Identification of the WDV strain spectrum in Saxony-Anhalt

Table 3 comprises all plants of different *Gramineae* so far tested for WDV strains with PCR. While in winter barley, oats, maize (inbred line FAP 1360 A and single cross hybrids) (FUCHS et al. 2001) and

Table 2. Infestation (%) of winter barley with WDV and BYDVs in autumn, spring, and early summer in the growth periods 1995/1996, 1998/1999, 1999/2000, and 1999/2000

Date of investigation	Growth period							
	1995/1996		1998/1999		1999/2000		2000/2001	
	WDV	BYDVs	WDV	BYDVs	WDV	BYDVs	WDV	BYDVs
Autumn	0.7 (0.0 ... 3.5)	49.0 (3.0 ... 80.5)	6.7 (0.0 ... 17.3)	0.9 (0.0 ... 3.3)	5.2 (0.7 ... 16.0)	0.2 (0.0 ... 0.7)	13.5 (5.3 ... 32.7)	5.8 (0.0 ... 10.0)
Spring	0.1 (0.0 ... 0.8)	24.0 (2.0 ... 91.2)	22.1 (10.0 ... 33.3)	3.0 (0.0 ... 18.7)	31.9 (10.0 ... 41.3)	0.7 (0.0 ... 2.0)	9.8 (3.3 ... 18.7)	30.4 (0.0 ... 69.3)
Early summer (new infections)	not tested		6.3 (0.0 ... 16.7)	0.3 (0.0 ... 2.0)	17.8 (0.7 ... 34.7)	0.3 (0.0 ... 1.3)	not yet tested	

also in the examined wild grasses only the barley strain was found, wheat housed also the wheat strain. An exception was triticale. Although the wheat strain was dominating (88.0 %), single plants of the same location might contain also the barley strain (12.0 %). There was no mixed infection of both strains in one plant.

### 3.4 Effectiveness of transmission of *Psammotetrix alienus* Dahlb.

Table 4 shows first results of the effectiveness of the vector *P. alienus* in transmitting WDV in different development stages. It seems that all development stages (L1 to L5 and imagines) are capable of transmitting the virus, yet with varying performance. As to WDV transmission, the virus acquisition by individuals of the L2-stage yielded the highest level (50.0 %, n = 12), followed by L3-stage (45.0 %, n = 20), L1-stage (42.9 %, n = 14), imagines (40.5 %, n = 42), L4-stage (23.6 %, n = 34) and L5-stage (9.1 %, n = 33). Anyhow, the different transmission mode of single individuals should be mentioned. Imagines, for example, include leafhoppers which are able to transmit WDV after acquisition continuously throughout an extended period. (e. g., animal No. 1: 100 days, No. 3: 85 d, No. 6: 80 d). Other leafhoppers, however, transmit WDV intermittently, which means that after successful infection more or less extended breaks are made (e. g., animal No. 11, animal No. 7). Sometimes, the first transmission was successful only 10 to 30 days after virus acquisition (e. g., animals No. 7, 17, 25). A similar situation was recorded in the different larval stages, but these experiments have not yet been finished. The results obtained so far agree with those gained by VACKE (oral communication).

### 3.5 Detection of viruliferous *P. alienus* in barley fields

The percentage of viruliferous individuals in a field population of *P. alienus* varied between approx. 80.0 % (June), 10.0 to 25.0 % (July to October) and about 40.0 % (November to December) (Fig. 2). Two leafhoppers were able to transmit the wheat strain in addition to the barley strain.

## 4 Discussion

The first evidence of WDV presence in Germany was provided by VACKE in 1990 (HUTH and LESEMAN 1994). However, earlier incidence is rather likely because mixing WDV up with BYDVs cannot be ex-

Table 3. Detection of barley and wheat strains of WDV in different host plants according to PCR

Host plants	Number of tested plants	Detection of	
		Barley strain	Wheat strain
Winter barley	330	330	0
Winter wheat	54	0	54
Oats	15	15	0
Triticale	43	5	38
Maize (inbreeding lines and single cross hybrids)	15	15	0
Species of different grasses	52	52	0

cluded because of equal or similar symptoms. In Saxony-Anhalt, comprehensive surveys of the occurrence of the virus have been made since 1995. They substantiate that in most years WDV can be easier identified in winter barley than BYDVs. The main attack takes place in autumn. According to MANURUNG et al. (2001), the vector *P. alienus* reaches a population peak in these months. In the same period, more than 40.0 % of all adults proved to be viruliferous. Moreover, infection sources in form of infected shatter grain and wild grasses were also existing. However, the relative share of WDV in plants with symptoms emerging from shatter grain shows no direct relationship to the infection of new sowings. This proportion is apparently related to the weather conditions in autumn. Warm and dry weather favour the vector. In autumn 1999, for example, the last living leafhoppers were caught in mid-November, in the following year only in mid-December. This indicates unanimously better and longer infection periods. According to MANURUNG et al. (2001), the summer of 2000 produced even three *P. alienus* generations, while it were only two in the preceding year. These relationships elucidate the high infection rate of winter barley (13.5 %, Table 1).

In the surveyed area, the first increased WDV attack in winter wheat and triticale was observed in autumn 2000. Each time, the fields had been sown rather early, i. e., in September.

Autumn sampling took place between middle and end of November. This time may entail further infection attacks. In order to record also these delayed infection processes, all controlled fields were again sampled in early spring, this included the time from the hatch of the first *P. alienus* generation. Usually, all infection rates had clearly increased in this case (for example 1998/99, 1999/00, Table 2). Only 2000/01 was an exception. So far, no explanation can be offered for this phenomenon.

Unexpected was the epidemic spreading of BYDVs in all winter wheat crops during the winter up to spring. In some cases, the infection rates reached > 80.0 %. Single fields were even ploughed under. WDV infection in autumn entailed the death of most plants during the winter up to early summer. Only single plants showed an infection latency in spring. Depending on the time of infection, the majority succeeded to develop one or more spike-bearing stalks. Now, the leave blades displayed chlorotic streaks.

Spring infection seemed to play a minor role (Table 2) apart from the year 2000 when by the month of June new infections had reached 17.8 %.

According to MANURUNG et al. (2001), the first leafhoppers appeared in mid-April of that year, which was approx. 3 weeks earlier than in 1999. In June, the portion of viruliferous leafhoppers was about 80.0 %. The majority of infected plants remained without symptoms, only few had chlorotic streaks on the blades of the youngest leaves. Yield losses are assumed to be low in case of spring infection.

The intense incidence of WDV in winter wheat and triticale in autumn 2000 underlines that both strains of WDV are present in the surveyed area. This was also proven by PCR sampling.

While in barley, oats and different wild grasses only the barley strain was detected, wheat seemed to house only the wheat strain. Only in triticale both strains were found.

The host range of WDV seems to be wider than assumed so far. Under natural infection conditions on the open ground, several new host plants of the genus *Bromus* (*B. arvensis*, *B. commutatus*, *B. hordeaceus*, *B. japonicus*, *B. sterilis*) as well as *P. arundinacea* were identified. Particularly, interesting might be the proof of WDV in the inbred line FAP 1360 A of *Zea mays* L. Comprehensive tests of other inbred lines and numerous maize cultivars showed in each case absence of the virus. Thus, in all probability a major susceptibility seems to exist in this line only.





Table 4 (Continued)

Number	Days after virus acquisition																					
	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	
23			X <sup>L5</sup>	X <sup>Im</sup>	X <sup>Im</sup>	X <sup>Im</sup>	+															
28		X <sup>L4</sup>	+																			
32		X <sup>L4</sup>			X <sup>L5</sup>	+																
L 5																						
6		O <sup>L5</sup>	O <sup>Im</sup>	X <sup>Im</sup>	O <sup>Im</sup>	O <sup>Im</sup>	+	O <sup>Im</sup>	O <sup>Im</sup>	O <sup>Im</sup>	+											
8	O <sup>L5</sup>		X <sup>Im</sup>	O <sup>Im</sup>	O <sup>Im</sup>	O <sup>Im</sup>		O <sup>Im</sup>	O <sup>Im</sup>	O <sup>Im</sup>			X <sup>Im</sup>	O <sup>Im</sup>	O <sup>Im</sup>	+						
25																						
Imago																						
1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	+
3	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	+
6	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	+
7	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
12	0	0	0	X	X	X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
14	0	0	X	X	X	X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
15	X	X	X	X	X	X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
24	0	0	X	X	X	X	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+	+
25	0	0	0	X	X	X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
26	0	0	X	X	X	X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
29	0	0	0	X	X	X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
33	X	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
34	X	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
35	X	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
41	X	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

X positive transmission

o no transmission

- experiment not finished

+ P. alienus dead

\* hopper is just alive

Im Imago

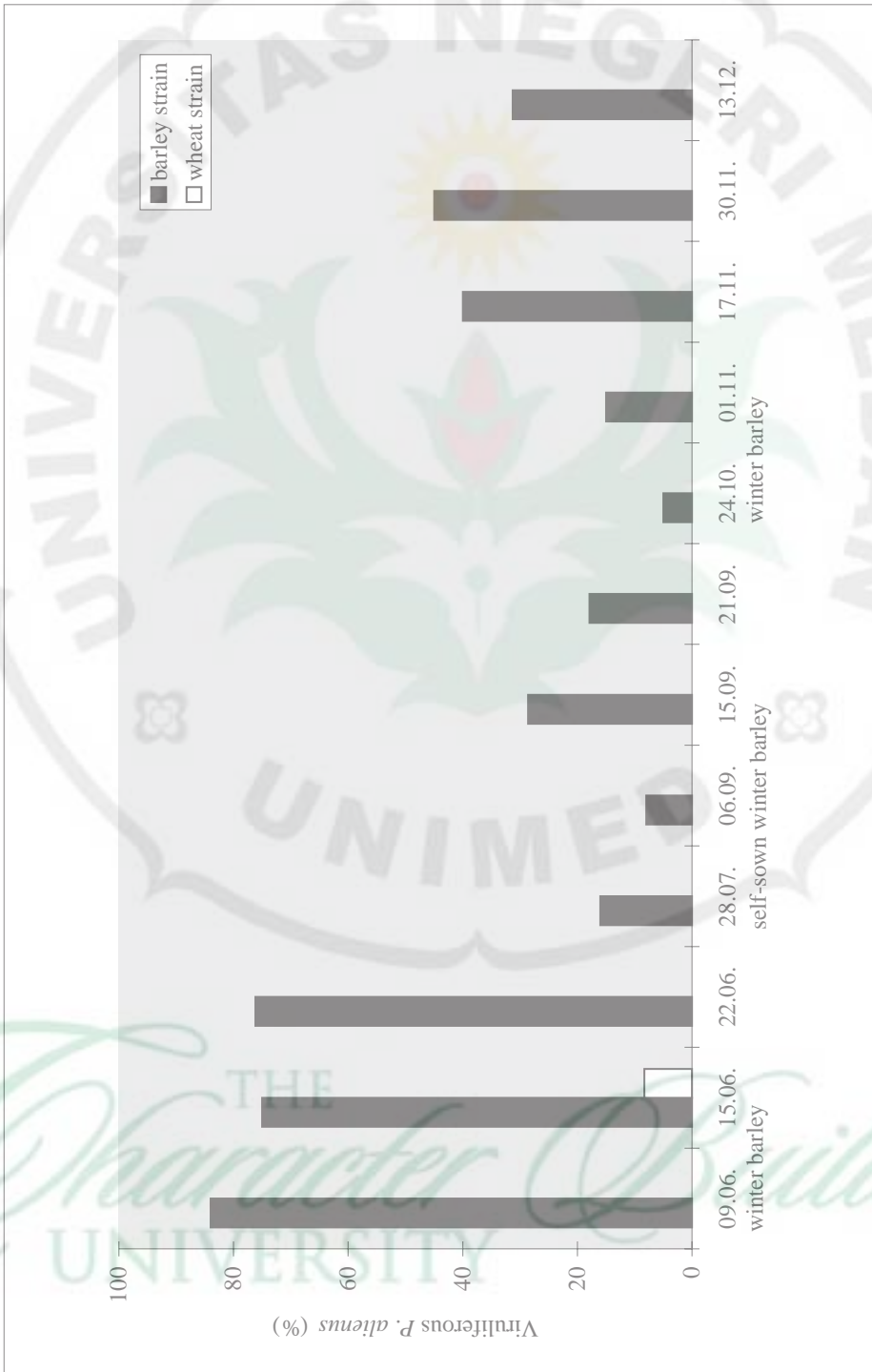


Fig. 2. Viruliferous *Psammotettix alienus* (%) in winter barley in Zscherben determined in a biological test

Obviously, the trait "susceptibility" is dominantly inherited in maize, because susceptible plants were also found in single cross hybrids containing the FAP 1360 A. As reported by competent maize breeders, the line FAP 1360 A has so far not entered any maize cultivar. This means that maize is a potential host plant of WDV indeed, but the currently grown cultivars become surely not infected.

By now, *P. alienus* has been regarded as the single vector of WDV although two other species of the genus *Psammotettix* (*P. confirmis* Dahlb., *P. helvolicus* Kbm.) and > 15 species of other genera have colonized cereal fields in Central Germany. Obviously, all larval stages and the imagines are potential transmitters, yet with varying effectiveness.

The imagines seem to have the greatest importance for WDV spreading within a crop, resulting from a life expectancy and increased mobility. Large differences in the individual transmission mode have been recorded between single specimens of all development stages; no explanation is available so far. The first determination of the percentage of viruliferous adults of *P. alienus* in the entire population on the open ground was made in 2000. Notable was the high number of viruliferous leafhoppers which varied strongly during the growth period. In autumn, approx. 40.0 % of all captured *P. alienus* adults turned out to be WDV transmitters. Thus, the population peak of leafhoppers coincides with the high incidence of viruliferous animals which offers ideal conditions for the infection of new sowings. The extent of actual infection, however, depends on the real weather conditions and the sowing date of the winter cereals.

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