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ORIGINAL ARTICLE

Comparison of two different experimental environments for resistance screenings for the leafhopper-transmitted wheat dwarf virus in wheat

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Abstract

Wheat dwarf virus (WDV) causes high yield losses in wheat and other cereals and is therefore an important pathogen transmitted by the leafhopper *Psammotettix alienus*. Climate change will increase infections by insect-transmitted viruses due to the increasing spread of vectors. In the context of integrated pest management, the cultivation of WDV-resistant/tolerant varieties is an effective way of controlling WDV. Evaluation of tolerant/resistant genotypes is based on inoculation with viruliferous leafhoppers and subsequent phenotyping in gauze houses under semi-field conditions. For successful screening, it is important to ensure the uniform and reproducible inoculation of plants. Abiotic conditions, particularly temperature, have a critical influence on inoculation success, and thus, variations in infection rates were observed within and between previous replicates in the field. Furthermore, the leafhopper population reared in the greenhouse has to be reestablished after each infection, which delays the screening process. We addressed these issues by developing an improved inoculation assay in which plants are inoculated in small infection hoods in the greenhouse before being planted out in gauze houses. This procedure allows optimal environmental conditions for WDV infection of test plants and allows the plants with WDV infection to develop under natural environmental conditions for symptom scoring. In addition, the viruliferous leafhoppers were recollected from the test plants after infection, allowing a sustainable use of the insects. The method thus enables more reliable phenotyping by increasing infection success and testing a greater number of genotypes in a shorter time.

Keywords Resistance breeding · Screening for resistance · Phenotyping · Plant virus

Introduction

The *Wheat dwarf virus* [WDV; family *Geminiviridae*, genus *Mastrevirus* (Fauquet et al. 2000)] infects a wide range of species of the family Poaceae, including economically important cereals such as barley, wheat, oat and rye (Vacke 1972; Lindsten and Vacke 1991; Vacke and Cibulka 1999). WDV was first described by Vacke in 1961 in the former Czechoslovakia (Vacke 1961; Lindsten and Vacke 1991) and later in other European countries (Bisztray et al. 1989). After its first report by Vacke (1961), WDV has become a problematic pathogen that poses a significant threat to wheat and barley production throughout Europe, Africa and Asia (Ramsell et al. 2008; Wu et al. 2008; Liu et al. 2012).

Symptomatic plants usually appear in patches in the field, and under permissible conditions entire fields may be infected, so that local epidemics can result in yield losses of up to 90% (Fohrer et al. 1992; Lindsten and Lindsten 1999; Lindblad and Waern 2002; Širlová et al. 2005). Symptoms of WDV infection include chlorosis, striping of leaves, shoot compression, reduced number of ears and stalks, sterile ears, delayed ear emergence, reduced winter hardiness and death of plants at an early developmental stage (Vacke 1972; Lindblad and Waern 2002). Symptom expression strongly depends on the stage of plant development at which the infection occurs. Infection at the two- to three-leaf stage causes plants to show more severe symptoms that negatively affect winter hardiness and yield. In contrast, infections that occur within the phase of stem elongation only lead to slightly shortened stems (Lindblad and Waern 2002). However, since the adults of the main vector species

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Psammotettix alienus are active in the field from April to December, the early stages of winter barley and winter wheat (Manurung et al. 2004; Mehner 2005) are most at risk.

The virus is transmitted in a persistent and circulating manner (Harrison 1977) by the leafhopper species *P. alienus* (Vacke 1961). Temperature appears to have a strong effect on the occurrence of *P. alienus,* as described by Manurung et al. (2004), who observed earlier and prolonged occurrences in the field during warmer years. In the fall, primary infection occurs after inoculation by adult leafhoppers, while secondary infection occurs in the spring through transmission by nymphs (Lindblad and Sigvald 2004). At temperatures above 15 °C, the insect population increases significantly, while temperatures below − 5°C cause death of the insects (Alla et al. 2001; Lindblad and Sigvald 2004; Manurung et al. 2005). In addition, temperature has an impact on virus transmission by *P. alienus*, with optimal conditions at 25 °C (Ghodoum Parizipour et al. 2016). Currently, only a few options are available to control the spread of WDV and related damage in the field. The most effective protection relies on agrotechnical and chemical measures to control the virus vector as a source of infection (Vacke 1989). The cultivation of tolerant/resistant varieties is an environmentally and climate-friendly alternative (Habekuß et al. 2009). However, phenotyping plants for tolerance/resistance to vector-transmitted viruses is difficult, e.g., due to the complexity of tritrophic interactions within the wheat (*Triticum aestivum*), leafhopper (*P. alienus*) and virus (WDV) complex. Furthermore, successful screening of resistant plant genotypes depends upon uniform inoculation of plants by WDV-carrying leafhoppers. In this study, we evaluated the efficacy of WDV infection by application of *P. alienus* from an in house rearing facility under two conditions: (i) Plants were cultivated and infested with *P. alienus* under semi-field conditions in a gauze house, and (ii) plants germinated and infected in the greenhouse. To evaluate our hypothesis that artificial conditions in the greenhouse improve WDV infection of a test set of plants, parameters such as germination rate, disease incidence and severity were examined comparatively for both setups..

Materials and methods

Leafhopper rearing

Leafhoppers of the species *P. alienus* were collected in 2018 in the field (51°30′21.3"N 11°45′39.1"E) and reared on WDV-infected wheat plants of the variety 'Alcedo'. They were kept in the greenhouse in Perspex® cages under controlled environmental conditions at 20 °C and a relative humidity (RH) of 60–70% with an 18 h light/6 h–dark period. Host plants were grown at 20 °C. Sunlight was supplemented by artificial lightning to maintain a 14 h light/10 h dark period. WDV infection of host plants in the rearing cages was regularly checked using a double-antibody sandwich–enzyme-linked immunosorbent assay (DAS-ELISA). For inoculation, leafhoppers were collected from the rearing cages using a custom-made exhauster.

Experimental design

Eighty genotypes consisting of the cultivars 'Hybnos', 'Faustus', 'Fisht', 'Moschus', 'Ponticus' and 75 susceptible and tolerant double-haploid (DH) wheat lines resulting from the F1 generation created by Strube Research (Söllingen, Germany) were used. DH lines were derived from crosses between the resistant genotype 'Fisht' and the susceptible genotypes 'Faustus', 'Moschus' as well as 'Ponticus'. Evaluation of resistant lines is based on the inoculation with viruliferous leafhoppers and a subsequent phenotyping for disease symptoms according to Vacke and Cibulka (2000). The two experimental procedures used for WDV infection are described below. All plants were treated with an insecticide (Pirimor G® (Adama, concentration 0.1%)) and a fungicide (Flexity® (BASF, concentration 0.1%)) as needed during the growth phase.

1st setup: outdoor inoculation in the gauze house

For resistance testing, gauze houses were used that protect plants against natural insect infestation and bird-induced damage. Plants were arranged in rows in a randomized block design with a row spacing of 20cm. Eighty lines with two treatments, (i) inoculated (WDV infected) and (ii) non-inoculated (non-infected control) arranged in opposite located rows—with twelve plants each in one row, were tested in a single gauze house (51°46′20.7″N 1°08′46.5″E). Plants were sown on 04.10.2018 (Fig. 1A). Two WDV-infected plants from the greenhouse were additionally planted per block as source of infection to increase infection pressure (according to Habekuß et al. 2009; Pfrieme et al. 2022). One block corresponds to five genotypes. An additional gauze tunnel system, steel arches covered with a double fleece with a mesh size of 0.39 × 0.88 mm (Ornata Plus 3988, Howitec, Bolsward, NL), was used inside the gauze house to separate the two treatments during the four-week period when the plants (treatment WDV-infected) were infected with viruliferous leafhoppers. At this point, most of the lines were at the beginning of tillering (BBCH 10). A stocking density of one leafhopper per plant was selected at a ratio of one nymph to four adults, and the insects were allowed to move freely inside the gauze tunnel. After the first frost, the gauze tunnel of the inoculated variant was removed and the plants were treated with an insecticide. One week later,

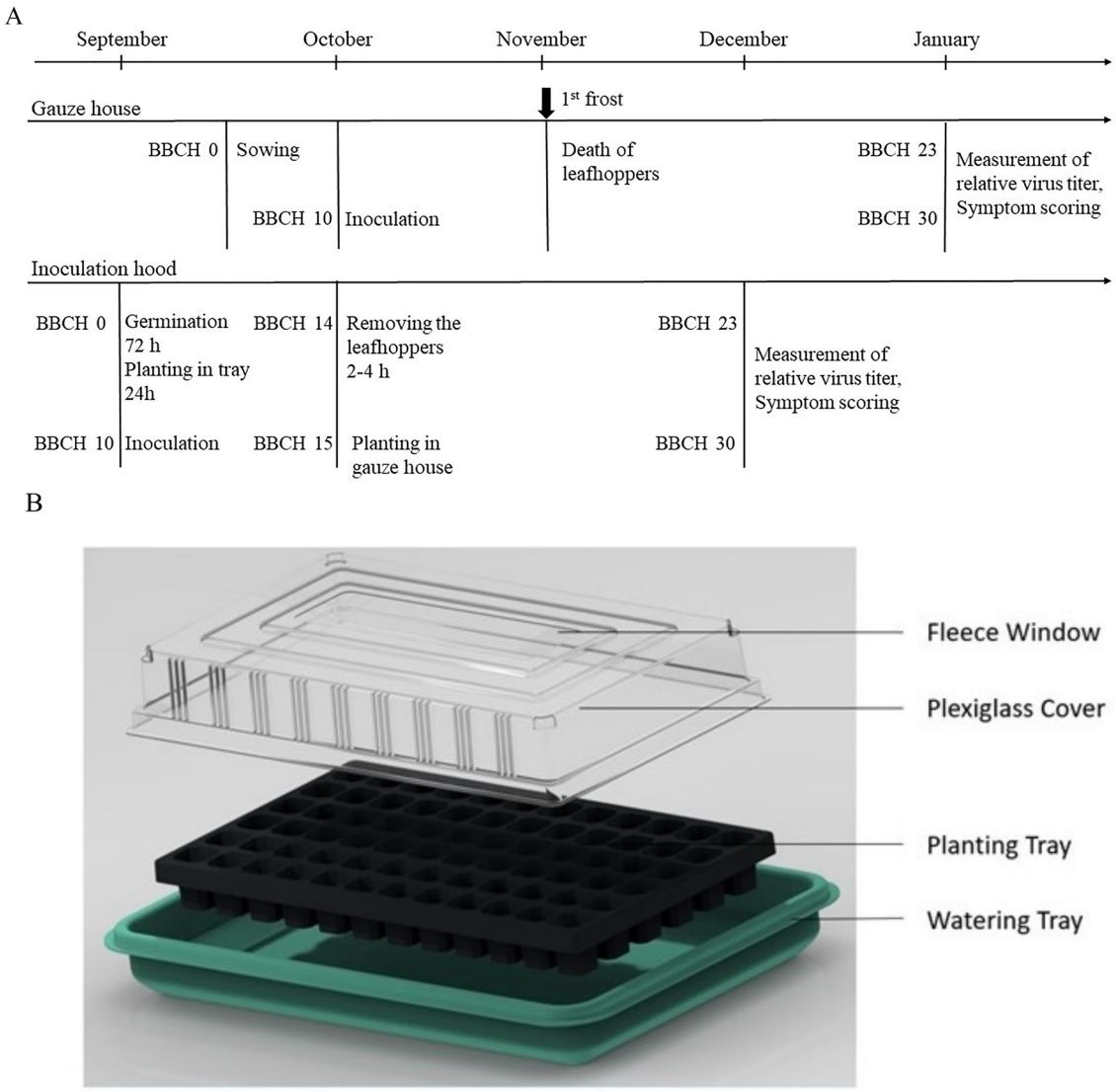

Fig. 1 **A** Flowchart of the workflow for inoculation in the gauze house (setup one) compared to inoculation under a hood in the greenhouse (setup two). Seeding in setup two occurred earlier. Traits' relative virus titers and symptom scores were recorded at the same BBCH stage. **B** Conception of an infection hood (model constructed by F. Jansen by using the software Creo parametric, PTC, version 5.0)

the gauze tunnel of the control treatment was removed and insecticide treatment was applied. For the field side, air temperature and humidity were measured at a height of 2m by a weather station (IMT 300, Pessl Instruments, Austria) located outside the gauze house. Air temperature (°C) and relative humidity (%) data were used to calculate daily mean, minimum and maximum values, and in accordance to the meteorological definition, air frost was defined as an air temperature below 0°C at a height of two meters (DWD 2021). Data recording stopped for setup one on the 39th day after inoculation following the onset of frost, as leafhopper activity and survival rate were expected to decline rapidly. Sampling to detect the virus infection using DAS-ELISA was carried out in January, 14 weeks after the first day of inoculation.

2nd setup: inoculation under an infection hood under greenhouse conditions

For the inoculation, which was carried out in setup two, planting trays of size 52 × 30 cm (Height x Width) with 7 × 12 planting holes (84 plants in total)) of size 4 × 4 cm (Height x Width) and a depth of 5 cm were used (Fig. 1B). Seven seeds from each line and variant were placed in containers and covered with an inoculation hood after a germination time of three days (Fig. 1A).

The planting trays were shielded with a 52.5 × 31.5 cm plexiglass cover into which a 44 × 20 cm fleece was glued. The seam of the lid was sealed with silicone to ensure that both the lid and the planting tray were closed tightly. Plants were watered through a tray (60 × 40cm with a depth of 7cm).

Thirty leafhoppers, 15 adults and 15 nymphs, were applied per planting tray. This corresponds to a density of one leafhopper per 2.8 plants, which is lower than in setup one to prevent overpopulation. Inoculation was performed over a 14-day period. During this time, the leafhoppers were encouraged to change location each day by exposing them to a random flow of air blown through the top fleece window. Containers were placed in the greenhouse under controlled environmental conditions at 16–32 °C and 18 h light/6 h dark at a relative humidity of 60–70%. Sunlight was supplemented by artificial lightning to maintain a 14 h light/10 h dark period. Containers were watered as required (according to Pfrieme et al. 2022).

The temperature was recorded every two minutes under the inoculation hood using a data logger (El-USB-1, Lascar electronics, Wiltshire, UK). Data recording was stopped for setup two, 15 days after inoculation, when leafhoppers were removed. Plants were treated with an insecticide. Control plants without WDV infection were equally treated. To check for WDV infection, a DAS-ELISA was carried out in December using material from the plants in the greenhouse.

Determination of germination rate

Germination was recorded for each plant line in both setups at the time of sampling for DAS-ELISA. Seeds were considered to be germinated when a plant was present. The germination rate was then calculated for the respective setup (Eq. 1).

$$\text{Rate of germination (I) (\%)} = \text{No. of seed germinated} / \text{No. of seeds sown} \times 100. \quad (1)$$

Determination of optimal inoculation time for the 2nd setup (inoculation hood)

To determine the optimal inoculation time, test plants were inoculated with leafhoppers for five, ten and 15 days. For each test, a single infection hood was prepared with the susceptible winter wheat variety ‘Alcedo’ with 77 plants each. All trials were conducted in parallel. Except for the inoculation time, experiments were conducted as described above. DAS-ELISA was performed six weeks after the first day of inoculation.

Assessment of WDV infection by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA)

In both experimental setups, the relative virus titer was determined from the infected variant by DAS-ELISA according to Clark and Adams (1977). Microtiter plates coated with specially prepared polyclonal antibodies (Julius Kühn-Institute, Quedlinburg/Germany) were used. Six weeks (inoculation hood) or fourteen weeks (gauze house) after inoculation, 50 mg of leaf material were collected from each plant of the infected variant.

The extinction value was measured photometrically at 405 nm, 30 min after addition of the enzyme substrate (p-nitrophenyl phosphate) using a microplate absorbance reader (Tecan Sunrise, Tecan, Männedorf, Switzerland).

The measured extinction value indicates the relative virus concentration. Leaf samples from healthy wheat plants (lyophilized leaf samples) were used as negative controls.

Positive infection was defined based on a calculated cut-off value for extinction (Eq. 2, Lardeux et al. 2016) in DAS-ELISA.

$$\text{Cut-off} = \text{mean}\bar{X}(\text{mean negative control}) + 3 \times \text{standard deviation of negative control} \quad (2)$$

Plants with an extinction value below the cutoff were defined as healthy and were excluded from the calculation of the mean relative virus titer.

Data analysis

Statistical tests were performed using R and R Studio, version 4.2.2 (R-Core-Team 2022). To analyze the data, a Shapiro–Wilk test for normal distribution and a Levene test (package ‘car’; Fox and Weisberg 2019) were first performed to determine homogeneity. Differences between the mean temperatures of inoculation conditions were tested using a Welch test (package ‘psych’; Revelle 2017). To determine optimal inoculation time, infection rates were compared pairwise with a chi-square test, and relative virus titer of infected plants were analyzed using ANOVA followed by Bonferroni’s post hoc mean comparison ($\alpha = 0.05$) (package ‘psych’; Revelle 2017). The correlation between the infection rate and the duration of inoculation, as well as relative virus titer, was determined using Pearson correlation coefficient test. A chi-square test was performed for the germination rate of the experimental setup, and the correlation between the experimental setup and the germination rate was determined by calculating the Pearson coefficient of correlation. The relative virus titers of WDV-infected plants in the experimental setups were compared using a Welch

test (package 'psych'; Revelle 2017), and a chi-square test was used to compare infection rates. Furthermore, a Pearson correlation coefficient test was performed between infection rates and experimental setup and between experimental setup and relative virus titer.

Results

Comparison of the inoculation conditions

To evaluate the influence of abiotic conditions on the success of the WDV infection, we recorded the temperature during the inoculation phase of the experiments (Fig. 2A).

Under controlled environmental conditions in the greenhouse where the infection hood was located, a daily mean temperature of 25 °C was selected for technical reasons, with an overall mean of 24.1 °C for the entire inoculation period and mean deviations of − 1.1 °C and + 1.15 °C (Fig. 2B). On all days, the mean temperature was above the threshold of 15 °C necessary for WDV transmission by leafhoppers and close to the optimal value for WDV transmission at 25 °C (Fig. 2A). During the inoculation period in the gauze house, a mean temperature of 9.5 °C was reached, with fluctuations of − 12.3 °C and + 8.6 °C. The threshold of 15 °C for daily mean temperature was reached only in the first four days, whereas the mean temperatures of days five, seven, and 33 were close to the 15 °C. During the period of days 12–26 and 35–39, even the maximum temperature was below the critical threshold of 15 °C. Temperatures below 0 °C were observed in the gauze house on day 22 and after day 35. Significant differences were found based on the daily average temperatures determined (Welch test, $t = -18.65$; $p < 0.01$), whereas setup two showed a higher mean temperature with less variations (Fig. 2B).

Determination of the optimal inoculation time

To test different inoculation periods for setup 2, we carried out inoculations for periods of five, ten and 15 days and determined the number of infected plants of the variety 'Alcedo' relative to the total number of plants (Fig. 3A) based on relative virus titer (Fig. 3B). Pairwise comparison of infected and non-infected plants using chi-square test showed that infection rates differed when inoculation occurred over five days ($n = 75$, infection rate 48%) and ten days ($n = 77$, infection rate 30%) ($\chi 2 = 4.52$; $p < 0.05$), five and 15 days ($n = 73$, infection rate 84%) ($\chi 2 = 19.17$; $p < 0.001$) and ten and 15 days ($\chi 2 = 41.69$; $p < 0.001$), with the highest number of infected plants detected 15 days after inoculation. Inoculation period did not affect relative virus titer (ANOVA, $F = 0.698$; $p > 0.05$; Fig. 3B). A low correlation was observed between the infection rate and duration of inoculation, based on a Pearson coefficient of correlation of $r = 0.41$ ($p < 0.01$).

Comparison of germination rate, disease incidence and severity

Due to the controlled conditions in the greenhouse (2nd setup), we expected better growth conditions when using infection hoods. For this reason, we determined the germination rate of infected plants for both experimental setups with twelve seeds (gauze house) and seven seeds (inoculation hood). The number of germinated plants for each genotype was recorded and related to the total number of plants/genotypes. Comparison of germinated and non-germinated plants using the chi-square test showed that the number of germinated plants was different when germination occurred in the gauze house ($n = 80$, average germination rate 70.42%) and under the inoculation hood ($n = 80$, average germination rate 84.64%) ($\chi 2 = 35.54$; $p < 0.01$), with the highest number of germinated plants detected under the inoculation hood. Based on the germination rate determined, a significant difference (t-test, $t = -5.19$; $p < 0.001$) was found between the two treatments (Fig. 4). The experimental design and germination rate were positively correlated (Pearson coefficient of correlation, $r = 0.38$; $p < 0.01$).

In order to compare infection data at the genotype level for both setups, we examined the number of genotypes that had at least one infected plant in both setups, in only one setup, or in no setup at all, using DAS-ELISA. About 58% of the 80 genotypes had at least one infected plant in both setups, whereas further genotypes showed infected plants in at least one of the two setups, and 20% showed no infection (Fig. 5A).

To determine the infection rates for the entire test population between the two setups, the number of infected plants was related to the total number of plants sown. Within the gauze house, twelve plants per line and treatment were raised, whereas only seven plants per treatment were sown under the inoculation hood. Comparison of infected and non-infected plants in the infected group by chi-square test showed that infection rates did not differ when inoculation was performed in the gauze house ($n = 669$, infection rate 31%) or under the inoculation hood ($n = 472$, infection rate 34%) ($\chi 2 = 0.95$; $p > 0.05$; Fig. 5B). Infection rate showed no significant correlation with the experimental setup (Pearson coefficient of correlation $r = -0.07$; $p > 0.05$).

In addition to the infection rate, the extinction value is also of interest for the determination of quantitative resistance. Therefore, we analyzed the relative WDV titers of the infected plants. Relative virus titers were significantly different between the two setups and tested for the sum of all

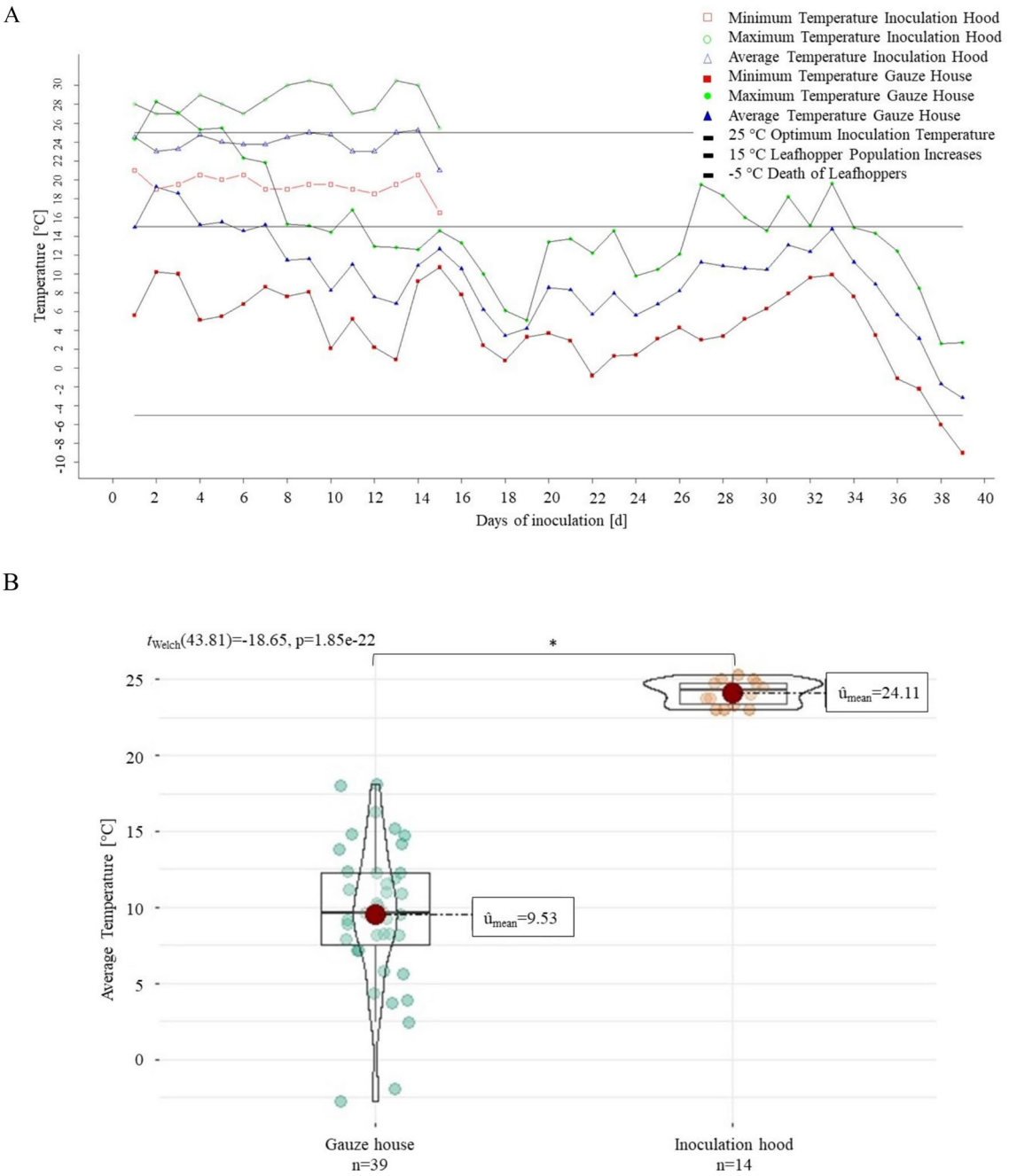

Fig. 2 **A** Temperature profile during the inoculation period. The mean minimum and maximum temperatures during the respective inoculation periods of setups one and two are given. The optimum temperature for WDV transmission was determined to be 25 °C. Temperatures above 15 °C provided optimal conditions for growing leafhopper populations. Temperatures < 0 °C led to reduced activity and temperatures < − 5 °C to death of the animals. **B** Average temperatures during inoculation in the gauze house and under the inoculation hood. Red dots indicate mean and black lines indicate median. Asterisks (*) mark significant differences ('ggstatsplot', Patil 2021). Test: Welch test; $p < 0.05$

positive infected test plants (Welch test, $t = -2.59$; $p < 0.01$; Fig. 5C), with a higher median for setup two. Setup and relative virus titers were positively correlated (Pearson coefficient of correlation, $r = 0.13$; $p < 0.01$).

Discussion

The objective of this study was to compare the efficiency of artificially released *P. alienus* regarding WDV infection of test plants in two experimental setups. In setup one,

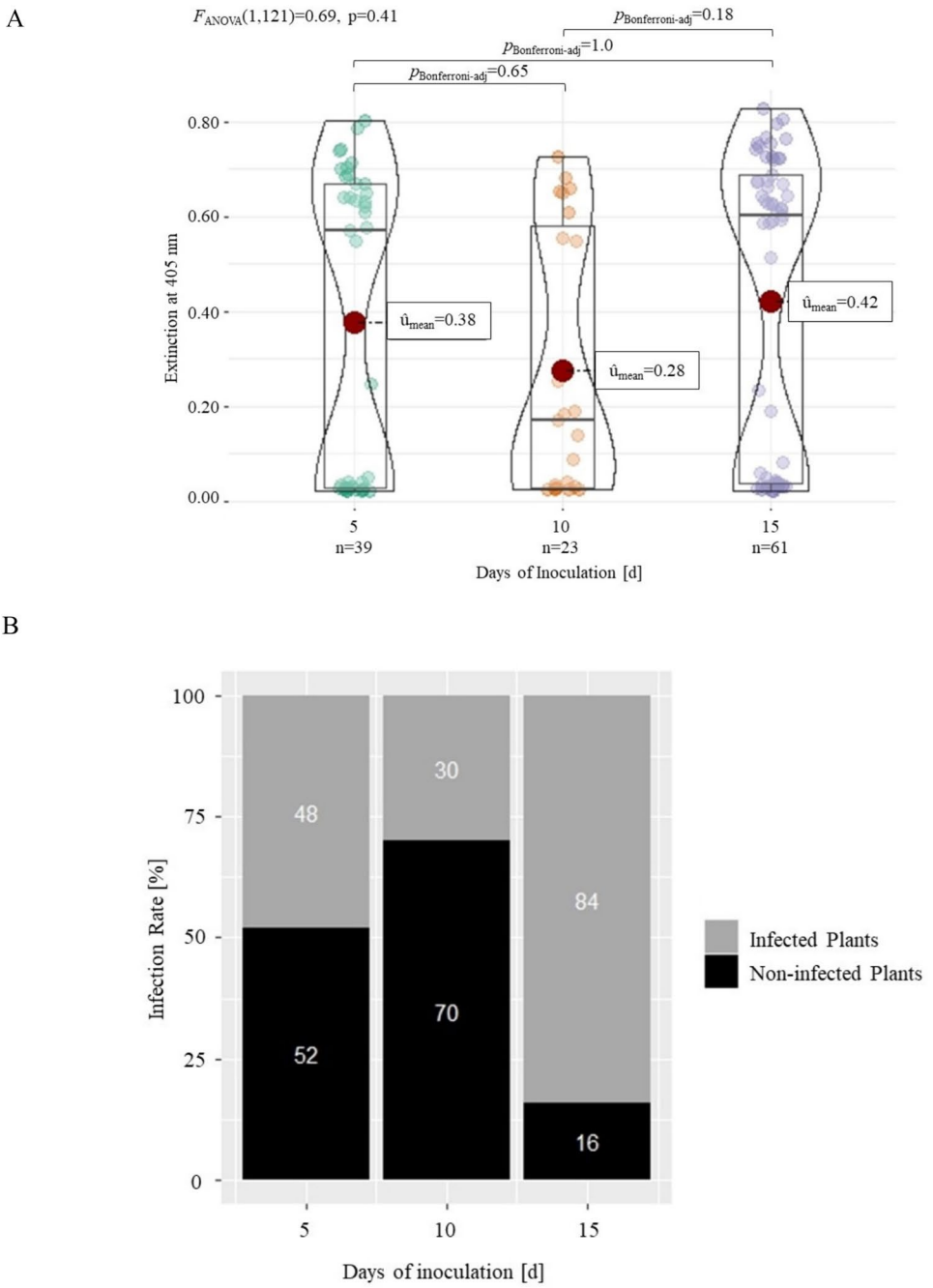

Fig. 3 **A** Evaluation of infection success after different inoculation periods. A total of 73–77 plants (5 d, $n=75$; 10 d, $n=77$; 15 d, $n=73$) were tested. These were classified as infected or non-infected. The percentage of plants determined is indicated on the y-axis (ggstatsplot, Patil 2021). B) Relative virus titer measured as extinction (405 nm) by DAS-ELISA of leaf samples of 'Alcedo' five, ten and 15 days after inoculation. The red dots indicate the mean and the black lines the median. Only infected plants were used for the analysis of the relative virus titer. $n=$ number of plants. Statistical test: ANOVA, Bonferroni $p<0.05$. The brackets show the results of the Bonferroni test

germination and inoculation occurred under semi-field conditions in a gauze house as used in previous studies (Habekuß et al. 2009; Pfrieme et al. 2022), whereas in setup two, germination and inoculation occurred under greenhouse conditions. In previous studies, experimental plants were individually inoculated in pots for the infection tests in the greenhouse (Banks et al. 1992; Scheurer et al. 2001; Niks et al. 2004; Nygren et al. 2015). However, this approach is space and labor intensive; therefore, we decided to use modified small plastic greenhouses as inoculation hoods. Although both methods are used, they have not been directly compared with each other.

Inoculation of WDV-transmitting leafhoppers on test plants under greenhouse conditions appears to have many advantages in terms of uniform inoculation, which is important for reliable and repeatable resistance screening of plant genotypes. In particular, leafhopper activity is temperature-dependent, with leafhopper population activity increasing at temperatures above 15 °C, and decreasing at temperatures below (Alla et al. 2001; Lindblad and Sigvald 2004; Manurung et al. 2005). Thus, fluctuations in infection rates

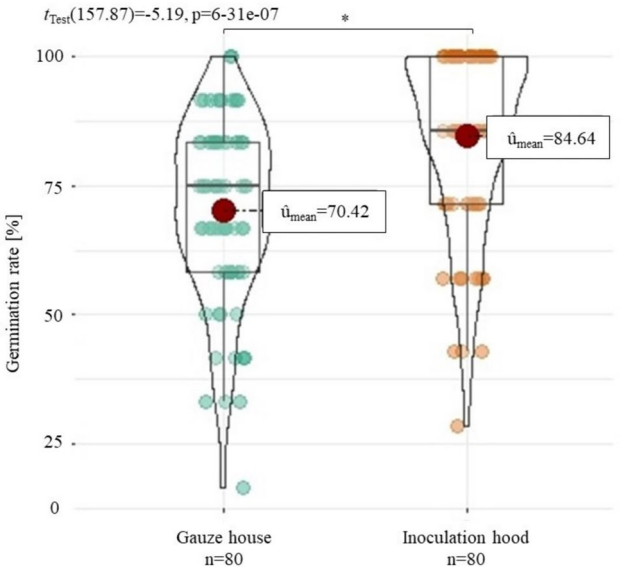

Fig. 4 Germination rate (%) of lines in two tested setups: gauze house and inoculation hood. The red dots indicate the mean values and black lines the respective median. n = Number of plants. Statistical test: t-test; $p < 0.05$. Asterisks (*) mark significant differences (ggstatsplot, Patil 2021)

may occur because of the temperature-dependent increased/reduced migration between test plants. Inoculation under greenhouse conditions should therefore lead to better infection rates, as the temperature never fell below 15 °C under our experimental conditions. In comparison, the conditions in the gauze house showed an overall lower mean temperature with greater fluctuations and a drop in the mean temperature below the critical value of 15 °C 8 days after infection, while the temperature of 0 °C was reached 22 days after inoculation. Studies have shown that the average daily maximum values under a gauze cover are 0.7–1.5 K higher than under field conditions and that this effect depends on the season and is strongest in the cooler months (Handelmann et al. 2001). However, the data indicate that leafhopper activity was likely limited under the conditions in the gauze house compared to setup 2, depending on the prevailing inoculation conditions at the time. These can vary from year to year and from location to location, resulting in a different duration of inoculation, which is important for successful infection with WDV, as has been shown in greenhouse trials.

Both setups are at risk of leafhopper egg overwintering on the test plants (Schiemenz et al. 1996; Nickel 2003), leading to uncontrolled WDV infection of plants from both treatments (WDV-infected and non-infected control plants) the following spring. Adult females lay eggs on plants from the beginning of August. While males die in September–October, females can be found until November–December (Witsack and Manurung 2005). Although we did not measure the number of eggs per plant for the two setups, we suggest that

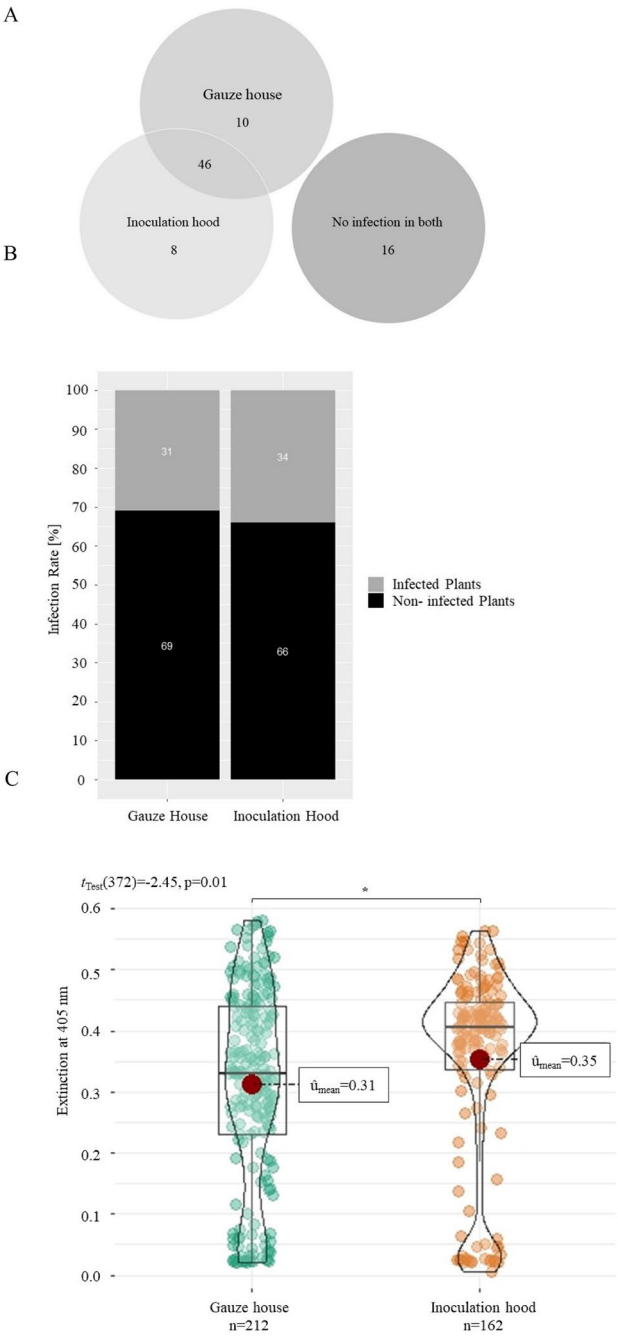

Fig. 5 Evaluation of infection success for setups with and without inclusion of an inoculation hood. Eighty genotypes were tested. **A** A Venn diagram displays how many of the genotypes tested were infected in both setups, in only one of the two setups, and showed no infection. **B** Evaluation of infection success after inoculation in two different environments: A total of 669 (gauze house) and 472 (inoculation hood) plants were tested. These were classified as infected or non-infected. The infection rate is indicated on the y-axis. **C** Relative virus titer measured using DAS-ELISA and shown as extinction (405 nm) in both setups. The red dots indicate the mean, and black lines indicate the median. Only infected plants were used for analysis. n = Number of plants. Asterisks (*) mark significant differences. Statistical test: t Whitney test; $p > 0.05$ (ggstatsplot, Patil 2021)

the risk for egg deposition is high for both setups, as in setup one, less optimal environmental conditions are accompanied by a longer incubation period, whereas setup two provides optimal environmental conditions with a reduced incubation period. Since both male and female adults were used, we assume that also females that contained mature eggs were included. For the measurement of the infection rate, which in our study is based on sampling in December or January, we can exclude an influence of newborn nymphs, as hatching is expected in spring. In contrast, the recording of agronomic traits could be influenced by following generations, which should be taken into account and could be managed by additional insecticide applications.

In addition to the temperature, the duration of inoculation might also be relevant for a uniform infection. Here, we did not observe a significantly reduced relative virus titer in infected plants between the test periods, but a longer inoculation period resulted in a significantly higher infection rate. Most likely, the higher infection rate can be explained by the greater number of plants visited and infected by the leafhoppers. For symptom-based resistance screening, it is important to note that relative virus titer is not necessarily related to symptom expression of the genotypes tested, as has already been shown for *Barley yellow dwarf virus* (Banks et al. 1992; Scheurer et al. 2001). However, the maximum inoculation periods tested here should be increased in future studies, as only 84% of the susceptible test plants were infected. Surprisingly, a difference in the number of days with optimal temperatures > 15°C has no effect on the number of infected plants and thus on the overall infection rate of all DH lines. We therefore assume that under semi-open field conditions, even short periods of activity, which can occur during temperature peaks, are sufficient to infect the test plants. Whether the difference in the number of infected DH lines occurred by chance or is due to a specific effect, such as the release of repellent components, cannot be said.

A comparison of the resistance screening with the 80 test lines revealed differences regarding the relative virus titers determined for both setups. Although setup one was inoculated over a longer period and samples were taken later than in setup two, the plants in setup two had higher relative virus titers. This confirms previous findings that environmental factors influence plant–virus interactions, as observed for the *Wheat streak mosaic virus*, where suppression of systemic spread to upper, uninoculated leaves occurs at temperatures below 15 °C (Seifers et al. 2013; Tatineni et al. 2016).

Significant differences in germination rates were observed within the study, with a higher germination rate achieved under greenhouse conditions in an inoculation hood. Seed germination depends on factors such as temperature, water, light, substrate and oxygen (Seo et al. 2009). A temperature sum of 90 °C and a minimum water content of 35–45% by weight are required for wheat germination. Excess moisture, which can occur during autumn, hinders germination by limiting the availability of oxygen. More optimal conditions probably exist under the inoculation hood, although they have not been determined except for temperature. Winter cereals germinate at temperatures between 3 and 37 °C, with an ideal temperature range of 12–25 °C (Agrifarming 2021). Before the onset of winter, plants should have reached the three-leaf stage (BBCH 13) to achieve good winter hardiness (Diepenbrock et al. 2016). These points are easy to control when plants are grown under greenhouse conditions. Overall, a better germination rate is especially beneficial when only a few seeds are available, which is often the case for breeding lines in early generations.

Conclusion

The experimental setups compared in this study represent suitable methods for WDV inoculation, as the minor differences between the two methods do not appear to affect resistance screening of plant population for plant breeding. However, both approaches have advantages and disadvantages from an experimental perspective. For example, for facilities without a greenhouse, inoculation in a gauze house is cost-effective and efficient. Nevertheless, the dependence of the infection of test plants on the weather should not be ignored, as it affects the mobility and survival of the vectors. The results presented here were obtained under warm autumn conditions. Consequently, observations over several experimental years are needed to determine the extent to which infection rates change under unfavorable weather conditions. For research questions based on phenotyping data, such as the detection of quantitative trait loci, the use of an inoculation hood followed by planting of experimental plants in the greenhouse may be advantageous. This further allows the recollection of leafhoppers from test plants, saving the effort and time required to propagate the insects and allowing for a higher throughput of experimental plants so that a greater number of genotypes can be tested. In this context, however, the subsequent planting of test plants in a gauze house should also be planned, as agronomic traits such as plant height and number of ears are necessary for a final decision on quantitative resistance and tolerance to WDV (Soleimani et al. 2023; Pfrieme et al. 2023).

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Availability of data and material The dataset presented in this study is deposited at OpenAgrar under DOI (not accessible during peer-review).

Declarations

Conflict of interest The authors declare no conflict of interest.

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