Research Article

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Temporal and species-specific resistance of sugar beet to green peach aphid and black bean aphid: mechanisms and implications for breeding

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Abstract

BACKGROUND: Sugar beet (*Beta vulgaris* ssp. *vulgaris*), a key crop for sugar production, faces significant yield losses caused by the black bean aphid *Aphis fabae* (Scop.) and the green peach aphid *Myzus persicae* (Sulzer), which also transmits viruses. The restriction on neonicotinoid usage in Europe has intensified this problem, emphasizing the urgent need for breeding resistant crop varieties. This study evaluated 26 sugar beet germplasms for resistance against both aphid species by using performance and feeding behavior assays. Additionally, whole plant bioassays and semi-field experiments were carried out with *Myzus persicae*.

RESULTS: Our findings demonstrate the presence of temporal resistance against both aphid species in the primary sugar beet gene pool. Beet yellows virus (BYV) carrying aphids showed enhanced performance. Different levels of plant defense mechanisms were involved including resistance against *Myzus persicae* before reaching the phloem, particularly in sugar beet line G3. In contrast, resistance against *Aphis fabae* turned out to be predominately phloem-located. Furthermore, a high incidence of black inclusion bodies inside the stomach of *Myzus persicae* was observed for approximately 85% of the plant genotypes tested, indicating a general and strong incompatibility between sugar beet and *Myzus persicae* in an initial phase of interaction.

CONCLUSION: Sugar beet resistance against aphids involved different mechanisms and is species-specific. The identification of these mechanisms and interactions represents a crucial milestone in advancing the breeding of sugar beet varieties with improved resistance.

Supporting information may be found in the online version of this article.

Keywords: electrical penetration graph; plant–aphid interaction; resistance screening; integrated pest management

1 INTRODUCTION

Sugar beet (*Beta vulgaris* ssp. *vulgaris* L.) is a globally cultivated important crop for sugar production, including its use in bioethanol. It is cultivated in over 50 countries and accounts for 20% of the world's sugar production.[1] Sugar beet production faces threats from herbivores, including planthoppers, nematodes, and aphids, resulting in significant yield losses.[2–4] Green peach aphid (*Myzus persicae* (Sulzer)) and Black bean aphid (*Aphis fabae* (Scop.)) are two major pests on sugar beet that serve as vectors for viruses, causing leaf yellowing, including beet yellows virus (BYV), beet mild yellowing virus (BMYV), beet chlorosis virus (BChV), and beet mosaic virus (BtMV).[5,6] *Myzus persicae* serves as the main vector as its transmission efficiency is higher. BYV is a phloem-located Closterovirus, which is transmitted semi-persistently (from hours to up to 3 days) by aphids.[7,8] Infection of BYV can cause sugar beet yield reductions of up to 47% due to leaf chlorosis or necrosis.[9,10] Since 2018, the European Union has banned the use of the three main neonicotinoid compounds, which were primarily applied for early sugar beet seedling protection against aphids.[11–13] The absence of alternative protective agents exacerbate issues in sugar beet production, leading to a decrease in the production area, total sugar output, and yield.[14]

Stringent pesticide restrictions challenge sugar beet farming. Genetic resistance research is vital for reducing pesticide use

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and achieving sustainability. In accordance with the International Organization for Biological and Integrated Control guidelines on Integrated Production (IOBC IP),[15] the selection of resistant/tolerant cultivars is necessary if available. Genetic resistance/tolerance has been identified against various pathogens, including fungal diseases[16,17] and nematodes, like the stem nematode *Ditylenchus dipsaci*[18] and beet cyst nematode *Heterodera schachtii* (Schmidt).[19,20] Studies regarding resistance against aphids[21,22] and BYV transmitted by *Myzus persicae*[23,24] were also conducted. Research that has investigated aphid resistance mechanisms in various crops, including Arabidopsis,[25] tomato,[26] pepper,[27] and cereal crops[28–30] has partially been used in plant breeding. These studies included for example, observations of aphid reproduction and survival[25] as well as the observation of feeding behavior by using the electrical penetration graph (EPG) technique.[27] The latter involves the detection of variations of a given electrical voltage, forming waveforms that can be allocated to different kinds of feeding behavior. The specific interaction between sugar beet and aphids is still poorly understood.[1] Transfer of aphids to sugar beet results in the formation of black deposits in their stomachs, which reduces aphid survival. This phenomenon has been observed in the aphid species *Myzus persicae*, *Aphis fabae*, *Macrosiphum euphorbiae*, and *Aulacorthum solani*.[31,32] BYV and BMYV infection can enhance aphid performance, reducing black deposit formation and mortality rate.[33] It was hypothesized that the black deposits in aphids are likely composed of proteins.[34] Mature plant resistance of sugar beets is involved as the black deposit formation rate is higher on older leaves. The black deposit development rate also varies significantly among six tested genotypes.[24]

The objective of this research was to screen a set of genotypes for resistance against the two aphid species *Myzus persicae* and *Aphis fabae*. The study employed behavior observation and fitness assays to differentiate between short- and long-term effects on genotypes and rank their resistance response. In addition to laboratory experiments, a semi-field trial was carried out to validate data from the laboratory in the field.

2 MATERIALS AND METHODS

2.1 Aphid rearing

Aphids were reared under controlled glasshouse conditions (20 °C, 16 h:8 h light/dark) at the Julius Kühn-Institut (Quedlinburg, Germany) to ensure parthenogenetic reproduction. Food plants were renewed every second week. *Myzus persicae* (collected from *Brassica oleracea* in Aschersleben, Germany in 1965) were reared on 4–6-week-old pepper '*Feher*' (*Capsicum annuum* L.) since then. The *Aphis fabae* population (donated by Wageningen University & Research from *Vicia fabae* in 1997) was maintained on 4–6-week-old sugar beet genotype *Sus* for 6 months prior to the experiments in *Vicia fabae*. If not specified in detail, reproductive apterous adults of random age were used for experiments.

2.2 Plant materials

A total of 25 sugar beet breeding lines (G1–G25) and one commercially available hybrid genotype (abbreviated as *Sus*) were provided by KWS SAAT SE & Co. KGaA (Einbeck, Germany). Seeds were germinated in 96-well plates with potting soil (Fruhstorfer Erde Typ T, Stocker Gartenbau UG, Schönau am Königssee, Germany). Individual seedlings were transplanted to an 8 cm × 8 cm × 8 cm pot filled with substrate (Einheitserde ED73, Gartenbautechnik Geereking, Hamburg, Germany). Plants were grown under glasshouse conditions (20 °C, 14 h:10 h light/dark). Plants with 5–6 true leaves unfolded at BBCH15–BBCH16[35] were selected for all experiments.

2.3 Leaf disk bioassay

Leaf disk bioassays were conducted for two aphid species using identical protocols. Ten replicates were analyzed in parallel for each genotype. An individual leaf disc with a diameter of 6 mm was cut from each plant and then placed inside a 35 mm × 10 mm Petri dish containing 2 mm 1.5% (*w/v*) tap water agar (Agar-Agar Kobe I, Carl Roth GmbH, Karlsruhe, Germany) including 0.03% (*w/v*) methyl-4-hydroxybenzoate (Sigma Aldrich, St Louis, MO, USA). The leaf disc's upper epidermis was attached to the partially solidified agar, and a single apterous adult aphid was placed on the lower epidermis near the midrib. The Petri dish was then closed, inverted, and placed in a climate cabinet for 48 h at conditions [20 ± 1 °C, 10000 Lux, 60% relative humidity (RH), 16 h:8 h light/dark) for 48-h synchronization. After the adult aphids produced the first instar nymph, all except one newborn nymph were removed to ensure age synchronization. The developmental stage of the nymphs was assessed every day. When reaching the reproductive stage, offsprings were counted and removed daily, and the monitoring was continued until the death of the last adult. Aphid population development was represented by the intrinsic rate of increase (r_m) (Eqn (1)) from Carey.[36] This value indicates the maximum population growth rate under the experiment, where l_x refers to day-specific survivorship and m_x refers to day-specific fecundity.

$$r_m = \frac{\ln(R_0)}{T} = \frac{\ln(\Sigma l_x m_x)}{\Sigma x l_x m_x} \quad (1)$$

Three sugar beet genotypes with the highest r_m-values (susceptible) for each aphid species and four genotypes with the lowest values (resistant) and *Sus* were chosen as potential candidates for further testing.

2.4 Black deposit formation

To investigate a genotypic effect of the 26 sugar beet genotypes on black deposit formation inside the aphids' stomach and to identify a potential correlation with aphid intrinsic rate of increase (r_m), leaf discs measuring 3 cm × 3 cm were cut from the fifth true leaf of each genotype and then placed on an agar-coated 60 mm × 10 mm Petri dish. For an experiment, ten apterous *Myzus persicae* aphids from capsicum were placed in a sugar beet leaf disc of a single genotype for 7 days in a climate cabinet (20 ± 1 °C, 10 000 Lux, 60% RH, 16 h:8 h light/dark). Six replicates were conducted for each genotype, and the number of aphids with black deposits was recorded at 7 DAI (days after infestation). The formation of black deposits was only studied for *Myzus persicae* as its green body color allows easier detection.

2.5 Feeding behavior observation with electrical penetration graph technique

Adult apterous aphids were randomly selected to monitor their 8 h-feeding behavior by EPG measurements.[37] *Myzus persicae* was tested on pre-selected G1, G3, G5, G9, G11, G17, G19 and *Sus*, *Aphis fabae* was tested on G3, G7, G11, G14, G17, G19, G22 and *Sus*. After 2 h of starvation, aphids were attached to an insect electrode connected to an EPG amplifier (EPG-Systems, Wageningen, The Netherlands). The plant electrode was inserted into the soil close to the sugar beet root. Aphids were placed on the fifth mature leaf. Data recording was done with the software EPG Stylet + d (EPG

Systems), and data were analyzed by visual checking[38–40] and labeling with the software EPG Stylet + a (EPG Systems). Detected waveforms are Np (non-penetration), C (stylet movement through the apoplast – probing), Pd (potential drop caused by cell penetration), E1 (secretion of watery saliva into SE), E2 (SE sap ingestion), G (xylem drinking) and F (stylet penetration problems). Incomplete recordings without waveforms at the first and last hour or with missing aphids on the insect electrode were excluded from the analysis. Twenty-seven EPG parameters were selected and calculated by using the Sarria EPG Workbook V4.4.3.[41] At least 20 replicates of each aphid-genotype combination were recorded.

2.6 Whole plant bioassay and DAS-ELISA

To investigate the effect of BYV on *Myzus persicae* population development on previously selected genotypes, each genotype was infested with either virus-infected (BYV) or virus-free (Control C) *Myzus persicae*. *Aphids of the species Myzus persicae* were transferred to either BYV-infected (Virus acquisition) or BYV-free detached *Sus* leaves (C) 2 days prior to the experiment. Subsequently, each plant of the BYV and C treatment received ten virus-carrying/virus-free *Myzus persicae*, respectively. The plant was then immediately covered with mesh plastic bags to prevent aphids from escaping. The plants were cultivated in a climate chamber (20 ± 1 °C, 10 000 Lux, 60% RH, 16 h:8 h light/dark) for 4 weeks. At 28 DAI, aphid populations were quantified. Following the count, the plants were sprayed with 0.028% (g/L) Tepekki (Belchim crop protection, Belgium) to remove the aphids. To confirm BYV infection, a total of 50 mg of leaf material was sampled from each plant, and a double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was performed by using the method described by Clark and Adams.[42] The polyclonal antiserum against BYV was obtained from LOEWE® Biochemica GmbH (Sauerlach, Germany). Plants were positively infected by BYV when the extinction value was above the calculated mean of the negative controls + 3 * SD (σ).[43] Plants with extinction values below the threshold were regarded as healthy. Data acquired from positive plants of the C-group and negative plants of the BYV-group were excluded from further analysis.

2.7 Semi-field test

To validate laboratory findings under field conditions, a semi-field test was conducted. Eight selected sugar beet genotypes with 20 replicates each were grown in the glasshouse and then transplanted to an open field with a gauze tent (ORNATA PLUS 3988, 24 m in length, 4 m in width, mesh size 0.39 × 0.88 mm) at BBCH15–BBCH16 stage. Tents protect plants from other wild pests and aphid predators. A total of 160 plants were randomly assigned into four columns and 40 rows with row width and column width of 0.5 m and 1 m, respectively. A 1 m wide path was set between column 2 and 3 for field management. Automated dripping pipes were installed for irrigation. After a 2-week plant adaptation period to the semi-field, ten aphids of the species *Myzus persicae* were placed on the fifth leaf of each plant, which was then covered with a mesh plastic bag measuring 35 cm × 12 cm. The utilization of mesh plastic bags with holes effectively facilitated controlled aphid colonization, protected against natural enemies, and enhancing aphid population monitoring and development. The bags allow gas exchange through small holes and were sealed with a metal strap at the bottom of the petiole to prevent the escape of the aphids. After 7 days, the plastic bags were removed, and aphids were allowed to move freely on the plant. The number of aphids per plant was counted at 15 and 30 DAI.

2.8 Statistics analysis

EPG parameters were compared among eight genotypes. Normality and homogeneity of EPG data were assessed by the Shapiro–Wilk test and Levene's test for each variable, respectively. Due to non-normally distributed data for all tested EPG parameters, all variables were analyzed by the Kruskal–Wallis test and Dunn's test for pairwise comparisons corrected by Benjamini–Hochberg. The independence of black deposit formation incidence of *Myzus persicae* feeding on each genotype was tested by Pearson's Chi-squared test. Correlation between black deposit rates and *Myzus persicae* r_m value at the corresponding genotype was examined by Spearman rank correlation test due to the absence of normal distribution of data. Significance levels were set at $P < 0.1$, $P < 0.05$, $P < 0.01$ and $P < 0.001$ (•, *, ** and ***, respectively). For whole-plant bioassay and semi-field data, aphid count was fitted into the count model. Due to the overdispersion caused by a large number of zero counts observed, the goodness of model fit was compared by Akaike information criterion (AIC) for the following five count models: Poisson model (P); negative binomial model (NB); zero-inflated Poisson model (ZIP); zero-inflated negative binomial model (ZINB); Hurdle model (H). After comparing the AIC, two models: (1) NB and (2) ZINB model (Eqns (2) and (3), respectively) were chosen for analysis.

$$\ln\left(\widehat{\text{counts}}\right) = \text{Intercept} + \text{b1I}(\text{Gei} = \text{G1}) + \ldots + \text{b8I}(\text{Gei} = \textit{Sus}) \quad (2)$$

$$\Pr(yi = j) = \begin{cases} \pi i + (1-\pi i)g(yi=0) \text{ if } j=0 \\ (1-\pi i)g(yi) \text{ if } j>0 \end{cases} \quad (3)$$

where πi represents the logistic link (the chance of finding zero counts and $g(yi)$ represents the log link (the *Myzus persicae* counts).

Statistical analysis was carried out by R version 4.1.3 in R studio.[44] Packages including 'FSA,'[45] 'pscl,'[46] 'car,'[47] and 'MASS'[48] were used.

3 RESULTS

3.1 Leaf disk bioassay

To evaluate the population development of the two aphid species on sugar beet genotypes, the intrinsic rate of increase r_m on all genotypes was calculated based on population data (Supporting Information Table S1) with an overall mean $r_{m(\textit{Myzus persicae})} = 0.66 \pm 1.01$ [mean ± standard deviation (SD), maximum 2.33, minimum −1.59] and $r_{m(\textit{Aphis fabae})} = 2.63 \pm 0.34$ (mean ± SD, maximum 3.4, minimum 1.9) respectively. The $r_{m(\textit{Myzus persicae})}$ values were negative on G1, G2, G3, G5, and G11 and all $r_{m(\textit{Aphis fabae})}$ were positive (Table S2). The r_m values for both species were plotted against each other (Fig. 1). Based on the extreme r_m-values for both species, four potentially resistant (R) and three susceptible (S) genotypes were selected respectively for each aphid species: R_*Myzus persicae* (G1, G3, G5, G11); R_*Aphis fabae* (G3, G11, G14, G22) and S_*Myzus persicae* (G9, G17, G19); S_*Aphis fabae* (G7, G17, G19). The populations of both aphid species developed fast on G17 and G19, and populations increased slowly or declined on G3 and G11.

3.2 Black deposit formation

When directly transferred from a capsicum plant without adaptation, the majority of *Myzus persicae* show formation of a black deposit in the stomach within 7 days. The average black deposit formation rate across all genotypes tested was 86.2% at 7 DAI. Pearson's Chi-squared test showed that the incidence of black

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deposit formation was significantly influenced by genotypes ($\chi^2_{(25)}$ =176.04, $P < 0.05$) with lowest rates on G8 (residuals = 3.73, 7.9%), G17 (residuals = 5.47, 17.0%), and G18 (residuals = 7.90, 35.5%) contributing the most differences between expected and observed values. Regarding the observed r_m value, genotypes G17 and G18 are relatively susceptible to both aphid species. No correlation was found between the $r_{m\ (Myzus\ persicae)}$ and the average black stomach deposit formation rate at 7 DAI ($r_{(df=24)} = 0.04$, $P = 0.83$) by Spearman's correlation test.

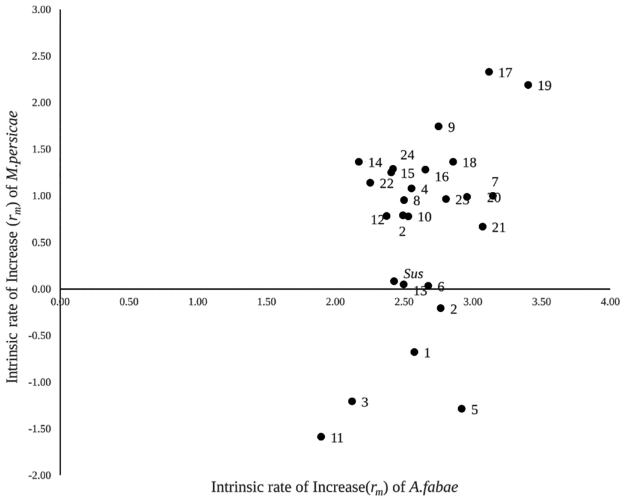

Figure 1. Scatter plot of the intrinsic rate of increase (r_m) for the two aphid species tested on 26 sugar beet genotypes. Data for *Aphis fabae* are plotted on *X*-axis and for *Myzus persicae* data are plotted on *Y*-axis.

3.3 Feeding behavior observation by EPG

For both *Myzus persicae* and *Aphis fabae*, 27 EPG parameters covering all tissues were analyzed to describe the latency to phloem contact/phloem feeding, total and mean duration of probing/non-probing/phloem feeding, and frequency of each feeding behavior (Table S3).

3.3.1 *Myzus persicae*

Six EPG parameters show substantial genotype-dependent differences (Table 1), specifically related to latency from either the 'start of recording' or 'first probing' to the first phloem phase. Dunn's test (Table 2) revealed significant differences ($P < 0.05$) between G3 and G9 for parameters 2, 3, 4, 5, and 6. G3 and G5 were statistically different ($P < 0.05$) for parameters 3, 4, and 6. Significant differences were also observed between G3 and G17 ($P < 0.05$) for parameters 4 and 6.

Table 1. Electrical penetration graph (EPG) parameters of feeding behavior show significant differences between eight genotypes

Aphid species	Tissue	EPG parameters	*P* Value	χ^2 (df = 7)
Myzus persicae	Epidermis and mesophyll	(1). start_1st E Time from start of EPG to 1st E	0.046*	14.60
		(2). 1st probe_1st E Time from 1st probe to 1st E	0.031*	15.69
	All tissues	(3). Start_1st sE2 (>10 min) Time from start of EPG 1st sustained E2 (>10 min)	0.041*	14.89
		(4). 1st probe_1st sE2 (>10 min) Time from 1st probe to 1st sustained E2 (>10 min)	0.028*	15.94
		(5). Start_1st E2 Time from start of EPG to 1st E2	0.046*	14.60
		(6). 1st probe_1st E2 Time from 1st probe to 1st E2	0.031*	15.69
Aphis fabae	Epidermis and mesophyll	(7). Aver_n(Pd)/probe Average number of Pd per probe	0.023*	16.23
	Phloem	(8). E1_in E% Contribution of E1 to phloem phase%	0.001***	15.69
		(9). T_dur_E Total duration of E	0.023*	22.11
		(10). T_dur_E1 Total duration of E1	0.042*	15.79
		(11). T_dur_E2 Total duration of E2	0.017*	16.29
		(12). M_dur_E1 Mean duration of E1	0.002**	14.59
		(13). M_dur_E2 Mean duration of E2	0.027*	15.79

Note: All 27 EPG parameters were compared between genotypes by Kruskal–Wallis tests and the parameters that show a significant difference are displayed.
*$P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Table 2. Pairwise comparisons of significant electrical penetration graph (EPG) parameters for *Myzus persicae* on eight selected genotypes

		(1). Start_1st E		(2). 1st probe_1st E		(3). Start_1st sE2 (>10 min)		(4). 1st probe_1st sE2 (>10 min)		(5). Start_1st E2		(6). 1st probe_1st E2	
Ge	N	Mean ± SD	SG	Mean ± SD	SG	Mean ± SD	SG	Mean ± SD	SG	Mean ± SD	SG	Mean ± SD	SG
G1	16	148.9 ± 116	a	147.6 ± 115.4	ab	172.4 ± 136.1	ab	171.1 ± 135.7	ab	164.1 ± 140.5	ab	162.8 ± 140.1	ab
G3	18	290.9 ± 146.9	a	289.8 ± 147.5	a	308.7 ± 150	a	307.6 ± 150.6	b	308.7 ± 150	a	307.6 ± 150.6	b
G5	16	142.7 ± 114.9	a	140.8 ± 115.6	ab	158.8 ± 128.4	b	156.8 ± 129.1	a	158.8 ± 128.4	ab	156.8 ± 129.1	a
G9	15	148.0 ± 123	a	142.5 ± 121.5	b	149.4 ± 123.1	b	143.9 ± 121.4	a	149.4 ± 123.1	b	143.9 ± 121.4	a
G11	20	205.9 ± 172.7	a	201.6 ± 171.7	ab	234.4 ± 176.5	ab	230.0 ± 175.9	ab	207.5 ± 171.6	ab	203.2 ± 170.7	ab
G17	17	165.6 ± 182.2	a	160.4 ± 180.8	ab	167.9 ± 180.7	ab	162.8 ± 179.4	a	167.9 ± 180.7	ab	162.8 ± 179.4	a
G19	15	194.1 ± 141.7	a	189.5 ± 135.8	ab	195.6 ± 141.3	ab	190.9 ± 135.4	ab	195.0 ± 141.6	ab	190.3 ± 135.8	ab
Sus	17	243.9 ± 183.8	a	241.7 ± 182	ab	246.5 ± 184.2	ab	244.3 ± 182.4	ab	246.5 ± 184.2	ab	244.3 ± 182.4	ab

Note: Mean value ± standard deviation (SD) in minutes is given for the six EPG parameters. A pairwise comparison was conducted by using Dunn's test. Different letters shown in the significant group (SG) column indicate statistically significant differences among genotypes based on adjusted *P*-value.

Table 3. Pairwise comparisons of significant electrical penetration graph (EPG) parameters for *Aphis fabae* on eight selected genotypes

		(7). Aver_n(Pd)/probe		(8). E_1_in E%		(9). T_dur_E		(10). T_dur_E1(s)		(11). T_dur_E2		(12). M_dur_E1		(13). M_dur_E2	
Ge	N	Mean ± SD	SG	Mean ± SD	SG	Mean ± SD	SG	Mean ± SD	SG	Mean ± SD	SG	Mean ± SD	SG	Mean ± SD	SG
G3	17	12.51 ± 8.5	a	2.4% ± 2.3%	a	216.0 ± 88.1	a	4.4 ± 3.8	a	211.6 ± 88.7	a	2.0 ± 0.8	abc	154.5 ± 111.8	a
G7	18	12.22 ± 6.1	a	7.3% ± 12.2%	ab	193.8 ± 119.8	a	8.1 ± 12.2	a	185.7 ± 119.4	a	4.5 ± 6.0	b	133.9 ± 121.2	a
G11	19	11.92 ± 10.3	a	5.4% ± 16.1%	a	235.5 ± 130.7	a	3.8 ± 3.2	a	231.8 ± 129.4	a	2.9 ± 3.4	abc	169.2 ± 130.8	a
G14	16	19.06 ± 8.1	a	5.4% ± 4.4%	b	167.9 ± 78.4	a	8.5 ± 6.8	a	159.4 ± 75.6	a	3.4 ± 2.7	ab	73.6 ± 51	a
G17	16	15.78 ± 8.6	a	4.5% ± 3.6%	ab	177.8 ± 101.4	a	5.4 ± 3.2	a	172.4 ± 101.6	a	1.4 ± 0.6	c	72.7 ± 83.6	a
G19	17	11.41 ± 8.6	a	2.5% ± 3.6%	a	251.4 ± 83.8	a	4.9 ± 5.3	a	246.5 ± 86.3	a	1.8 ± 1.1	a c	143.4 ± 115	a
G22	16	12.2 ± 7.1	a	8.0% ± 8.6%	b	153.1 ± 96.9	a	7.5 ± 5.0	a	145.7 ± 96.9	a	3.3 ± 3.6	abc	89.5 ± 100.5	a
Sus	19	14.65 ± 8.8	a	3.2% ± 3.1%	ab	248.6 ± 90.3	a	7.9 ± 10.2	a	240.8 ± 88.4	a	2.9 ± 5.1	a c	116.9 ± 102.5	a

Note: Mean value ± standard deviation (SD) in minutes or percentages is given for the seven EPG parameters. A pairwise comparison was conducted by using Dunn's test. Different letters shown in the significant group (SG) column indicate statistically significant differences among genotypes based on adjusted *P*-value.

3.3.2 *Aphis fabae*

Seven EPG parameters showed genotype-dependent differences (Table 1). These parameters were related to sieve element-associated activities and cell penetration. Dunn's test (Table 3) revealed significant differences ($P < 0.05$) between G14 and G11/G19 for parameter 8, between G22 and G3/G11/G19 for parameter 8, between G7 and G17/G19/*Sus* for parameter12, and between G14 and G19 for parameter 12.

3.4 Whole plant bioassay

To investigate *Myzus persicae* population development on selected eight sugar beet genotypes and the potential effect of BYV, a total of 160 plants were checked for aphid number in the glasshouse (mean 10.94, median 0, maximum 144, minimum 0). Of these, 118 plants remained uncolonized by *Myzus persicae* at 28 DAI (Table S6 and Fig. S1). When comparing different models, the best-fitted model was the zero-inflated negative binomial (ZINB) model with a goodness-of-fit indicated by the lowest AIC value of 561.67. This model included the eight genotypes and treatments as covariates (Table S4).

When compared to *Sus*, exponentiated coefficients of G5 [odds ratio (OR) = 7.16, $z = 2.48$, $P = 0.01$], G9 (OR = 25.27, $z = 2.85$, $P < 0.01$) and G17 (OR = 5.27, $z = 2.25$, $P = 0.02$) show a significant difference (Table 4). G1 ($\beta = 0.28$, $z = -2.67$, $P < 0.01$) and G3 ($\beta = 0.11$, $z = -3.85$, $P < 0.01$) were significantly associated with a decreased number of counts while G19 ($\beta = 3.74$, $z = -3.364$, $P = 0.02$) was associated with a significant increase in aphid counts. G19 showed a high intrinsic rate of increase for *Myzus persicae* and a lower formation of black deposits in contrast to G1 and G3, whose intrinsic rate of increase was low for *Myzus persicae*. Compared with BYV-inoculated aphids, the counts of aphids in the control group (C) were significantly lower ($\beta = 0.21$, $z = 2.85$, $P < 0.001$).

Table 4. Zero-inflated negative binomial (ZINB) model coefficients of *Myzus persicae* at 28 DAI (days after infestation)

	Day 28 (ZINB model)	
Genotypes	Zero inflation model	Count model
Sus	0.64	68.26***
G1	2.13	0.28**
G3	3.90•	0.11***
G5	7.16*	0.48
G9	25.27**	1.43
G11	3.85•	0.66
G17	5.27*	1.07
G19	2.48	3.74*
Treatment C	1.33	0.21***

• $P < 0.1$.
*$P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Table 5. Negative binomial (NB) model coefficients of *Myzus persicae* at 15 DAI (days after infestation) and zero-inflated negative binomial (ZINB) model prediction of coefficients at 30 DAI

	Day 15 (NB model)	Day 30 (ZINB model)	
Genotypes	NB model	Zero inflation model	Count model
Sus	0.65	1.71	13.29***
G1	8.08**	0.44	2.35
G3	2.46	0.27	2.14
G5	8.69**	0.17*	3.92*
G9	2.08	0.35	2.39
G11	1.38	0.26•	1.97•
G17	5.08*	0.12*	3.51•
G19	13.20***	0.14*	5.34**

• $P < 0.1$.
*$P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

3.5 Semi-field test

One of the G19 plants died after transferring them to the semi-field and therefore a total number of 159 plants were monitored. Data were analyzed separately for 15 DAI and 30 DAI (Tables S7 and S8, and Fig. S2). At 15 DAI, 93 zero aphid counts were observed (mean 3.38, median 0, maximum 83, minimum 0), and at 30 DAI, 63 zero aphid counts were observed among all plants (mean 26.84, median 4, maximum 475, minimum 0). A comparison of goodness-of-fit by five models was conducted to examine the best-fitted model (Table S5). The NB model was selected at 15 DAI (AIC = 584.05), and the ZINB model was selected at 30 DAI (AIC = 652.22) based on the lowest AIC value to examine the genotypic effect on aphids counts.

For 15 DAI, when compared with *Sus*, exponentiated coefficients of G1 ($\beta = 8.08$, $z = 2.84$, $P < 0.01$), G5 ($\beta = 8.69$, $z = 2.94$, $P < 0.01$), G17 ($\beta = 5.08$, $z = 2.19$, $P = 0.02$) and G19 ($\beta = 13.20$, $z = 3.48$, $P < 0.01$) had significantly increased counts (Table 5). G19, as previously selected as one of the susceptible genotypes, has the highest aphid estimates among all genotypes.

For 30 DAI, when compared with *Sus*, exponentiated coefficients of G5 (OR = 0.17, $z = -2.06$, $P = 0.04$), G17 (OR = 0.12, $z = -2.21$, $P = 0.03$) and G19 (OR = 0.14, $z = -2.17$, $P = 0.02$) shows significant difference (Table 5). Indicating the odds of zero counts were significantly lower on these genotypes than that of *Sus*. G5 ($\beta = 3.92$, $z = -2.06$, $P = 0.03$) and G19 ($\beta = 0.11$, $z = -3.01$, $P < 0.01$) were associated with a significantly increased number of aphid counts.

4 DISCUSSION

Resistance against aphids and yellowing viruses is crucial for sugar beet farming due to restrictions on neonicotinoid application. The screening of 26 sugar beet genotypes (25 lines, one hybrid), under laboratory and semi-field conditions, demonstrates that resistance against *Myzus persicae*, and strong differences in susceptibility against *Aphis fabae* respectively is present within the primary gene pool of *Beta vulgaris* ssp. *vulgaris*. Some genotypes even showed resistance/low susceptibility properties to both aphid species. These genotypes can be easily integrated into breeding programs.

The intrinsic rate of increase (r_m) was utilized to identify aphid resistance among sugar beet genotypes. The parameter r_m measures population growth in the absence of predators[49] and is widely used in laboratory experiments. Due to the short

generation time and higher reproduction of aphids, a high r_m-value can be estimated for susceptible genotypes. For *Myzus persicae*, r_m-values range for different host species between 0.2–0.3[50,51] and 0.46.[52] Under glasshouse conditions, *Aphis fabae* r_m-values range between 0.13 and 0.45 for sugar beet[53,54] and between 0.14 and 0.35 for other host plant species.[50] In our study, r_m-values of *Aphis fabae* were higher than reported, probably due to previous adaptation to sugar beet, indicating the susceptibility of all genotypes tested. The r_m-values for *Myzus persicae*, however, showed both positive and negative values. No specific r_m data for *Myzus persicae* on sugar beet have been found in the literature. The switch of the host plant may have a beneficial effect on r_m-values as reported by Fernandez-Quintanilla *et al.*,[50] who observed an increase after a switch of the host plant. In our experiments, the performance of both aphid species indicates different levels of susceptibility and resistance within the genotypes tested.

Feeding behavior analysis for the selected genotypes by EPG revealed that *Aphis fabae* and *Myzus persicae* respond differently to plant defense mechanisms, leading to a potential decrease in nutrient intake, and as a result, a decline in population growth. However, only *Myzus persicae* feeding on the genotype G3 exhibited significant changes in feeding behavior related to plant resistance. This was characterized by an increased duration from the beginning of probing to first phloem-associated behavior (1st E) and ingestion (1st E2) as well as sustained ingestion (1st sE2). This increase in duration implies strong incompatibility between *Myzus persicae* and G3. No comparable observations were found for G1, G5, and G11, which were designated as resistant based upon observed negative r_m-values. Pathway activities include the intercellular stylet movement and penetration of epidermal and mesophyll cells including cell sap sampling, which are required for host–plant identification and acquiring cues for feeding.[55–58] Hereby, aphids are confronted with plant defense mechanisms at different cell layers probably impairing fitness.[59,60]

Although pathway activities vary in *Myzus persicae* at distinct genotypes in Arabidopsis[61] and potato[62] apoplast-located plant defense mechanisms also result in a prolonged pathway phase, such as ADF3-dependent mechanism and ascorbate peroxidase activity.[60,61,63]

For *Aphis fabae*, EPG results show a predominant effect on phloem-located parameters, particularly on G14 and G22. This is reflected in a higher proportion of E1 in E and an increased duration of salivation and decreased ingestion, but the latter is not significant in comparison to other genotypes. An increase in the secretion of saliva into sieve elements acts as a counter-measure to suppress sieve element-located defense mechanisms by using effectors, for example, by Will *et al.*[64] Sieve element defense mechanisms are widely studied in plant–aphid interactions, comprising a variety of mechanisms, such as structural P-proteins like sieve element occlusion proteins.[65–67] Others involved in plant defense against aphids are, for example, NBS-LRR receptors, present in the melon genotype TGR-1551,[68] and chaperons described for Arabidopsis.[69] Regarding the r_m-values, low susceptibility against *Aphis fabae* does not necessarily correlate with significant changes in feeding behavior, which was also observed for *Myzus persicae*-genotype interactions. Surprisingly, genotype G3 negatively affects both aphid species as a resistant genotype against *Myzus persicae* and less susceptible to *Aphis fabae*. The observed impact on the feeding behavior of *Myzus persicae*, but the absence of a comparable effect on *Aphis fabae*, suggests the occurrence of specific plant genotype-aphid species mechanisms during short-term interactions (8 h), as reported by Williams *et al.*[70] Additionally, generic mechanisms are effective, bringing long-term effects on population growth but the two aphid species appear to vary in their respective sensitivity. Phloem-located phenolics with antimicrobial activity can negatively impact bacterial endosymbionts of aphids like *Buchnera aphidicola* resulting in reduced fitness.[71] Polyphenols present in sugar beet molasses and originating from whole plants have antimicrobial effects, for example, against *Escherichia coli*.[72] Phenol content is also increased in some wheat genotypes as a defense response to aphids' infestation.[73] Other metabolic compounds may also be involved, but further data are needed.

The generalist aphid *Myzus persicae* exhibited lower efficiency in colonizing sugar beet. A two-fold incompatibility is hypothesized for this low colonization efficiency: unsuccessful colonization and inhibited population development. In our semi-field experiment, few aphid populations exceeded ten individuals at 15 DAI. We assume that the majority of the *Myzus persicae* observed at 15 DAI were the adults that initially infested the respective plants, as our leaf disc tests showed that it usually takes 10–15 days for nymphs to reach adulthood on sugar beets while new-borne progeny were rarely spotted during field surveillance. The high likelihood of zero counts at 15 DAI can be attributed to the unsuccessful infestation by aphids, likely due to plant genotype effects. At 30 DAI, the large proportion of the *Myzus persicae* counts greatly outweighed the population sizes at 15 DAI, suggesting the presence of new nymphs. At 30 DAI, the large proportion of the *Myzus persicae* counts greatly outweighed the population sizes at 15 DAI, suggesting that the new nymphs already reached maturity. Thus, the counts observed on colonized plants reflect genotype effects on aphid population development. Initial incompatibility between *Myzus persicae* and sugar beet is universal, as indicated by the absence or very low abundance of aphids on sugar beet plants. This incompatibility is particularly pronounced in resistant genotypes G3 and G11, with consistently low aphid counts in both whole plant assays and semi-field trials. In contrast, populations of *Myzus persicae* preferentially established on susceptible candidates G17 and G19. The ZINB model, previously used in invasive pest control studies by, e.g. Kamiyama *et al.*[74], helps to understand the relationship between zero counts and monitoring parameters, distinguishing absence from imperfect monitoring. Our research highlights the nested ZINB model providing insights into how the factor genotype influences colonization and population growth. This model is an invaluable tool for identifying resistant genotypes with moderate to high plant–pest incompatibility.

Black deposits in the stomach contribute to initial population declines, as their formation leads to the rapid death of aphids,[24,75] in both whole plant assay and semi-field trial as an indicator of plant resistance in sugar beets. Furthermore, 7 days after the transfer of *Myzus persicae* from capsicum to detached sugar beet leaves, black deposits were present in over 85% of the aphids in all genotypes tested. This indicates a general defense mechanism in sugar beets that leads to the deposition of substances inside the aphid's stomach, resulting in subsequent lethal effects. As demonstrated for *Myzus persicae*, the rate of black deposit formation is not statistically correlated to resistance classification based on $r_{m(Myzus\ persicae)}$, and varies among genotypes. The lack of correlation suggests an adaptation process against this mechanism, which allows the aphids to establish on sugar beet. This adaptation may involve the expression of detoxification enzymes in the salivary glands or stomach, and/or a preference for colonizing younger leaves, as black deposit formation

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is more common on older leaves.[24] BYV infection enhanced aphid life history in the whole plant assay, resulting in larger populations at 28 DAI for most genotypes, particularly G19. With a high average rate of black deposit formation, and low r_m-value in BYV-free plants, it can be suggested that BYV infection suppresses plant defense mechanisms and by doing so reduces the mortality of *Myzus persicae*, supporting the finding of Kift *et al.*[33] As BYV-associated effects were only observed in the whole plant assay where black deposit formation was not examined, no final conclusions can be drawn.

We summarize that the tested lines exhibited varying levels of resistance to aphids, indicating the involvement of different temporal levels of resistance and resistance mechanisms. From a plant breeding perspective, the observed long-term mechanism could be valuable in keeping populations of *Aphis fabae* permanently low, while the mechanism causing the black deposits could suppress the spread of incoming individuals of the species *Myzus persicae* and thus the spread of BYV. Further investigation is required to understand the background as well as the potential adaptation to this mechanism. Since a virus infection could be used as a putative tool by aphids to enhance genotype accessibility, as suggested by Kern *et al.*[43] for BYDV and *Rhopalosiphum padi*, the absence of such an effect seems to be a trait for plant breeding, highlighting the need for detailed investigation of this mechanism.

AUTHOR CONTRIBUTIONS

YZ contributed to the design and implementation of the research as a part of his PhD work. YZ and TW conducted experiments. YZ and TW analyzed data. The first draft of the manuscript was written by YZ. All authors TW, MR, and AS reviewed and edited the manuscript. All authors read and approved the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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