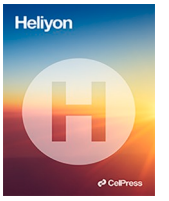
Heliyon 10 (2024) e32429

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Facilitation of *Sclerotinia sclerotiorum* infestation by aphid feeding behaviour is not affected by aphid resistance in oilseed rape

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A R T I C L E I N F O

Keywords:
Brevicoryne brassicae
Sclerotinia sclerotiorum
Oilseed rape
Feeding behaviour
The electrical penetration graph

A B S T R A C T

The relation between aphids and Sclerotinia stem rot (SSR) in oilseed rape is rarely examined because they are often studied alone. We have observed a significant correlation between the number of aphids and the occurrence of SSR in our field studies. Electropenetrography (EPG) was used to evaluate the effects of *Brevicoryne brassicae* (Linnaeus) on two oilseed rape cultivars while acquiring, vectoring and inoculating of *Sclerotinia sclerotiorum* Lib. (de Bary) ascospores. The results demonstrated that aphid feeding followed by the application of an ascospore suspension significantly increased *S. sclerotiorum* incidence. Aphids were capable of adhering to ascospores and carrying them to healthy plants, thereby causing diseases. The results of the EPG analysis indicated that aphid feeding behaviour was significantly altered in all leaf tissue levels following infection with *S. sclerotiorum*. Aphids initiated their first puncture significantly sooner than the control group, began probing mesophyll cells earlier, significantly increased the frequency of both short probes and intracellular punctures and had a significantly shorter pathway duration. On infected aphid-susceptible cultivars, aphids secreted more saliva but had reduced ingestion compared with aphids feeding on non-infected oilseed rape. In addition, ascospores can affect aphid feeding behaviour by adhering to aphids. Aphids carrying ascospores punctured cells earlier, with a significant increase in the frequency and duration of short probes and cell punctures, shortened pathway durations, increased salivation and reduced ingestion compared with aphids not carrying ascospores. On aphid-susceptible cultivars, aphids carrying ascospores delayed puncture onset, but on resistant cultivars, puncture onset was shortened. There is a correlation between aphids and *S. sclerotiorum*. The impact of *S. sclerotiorum* on aphid feeding behaviour is directional, favouring the spread of the fungus. This promotion does not appear to be altered by the aphid resistance of the cultivar.

1. Introduction

Sclerotinia stem rot (SSR) is caused by the necrotrophic pathogen *Sclerotinia sclerotiorum* Lib. (de Bary) and attacks cotyledons and leaves in seedlings, as well as stems and leaves in adult plants. The symptoms of SSR include water-soaked lesions, necrotic tissues with fluffy white mycelia and sclerotia inside the stems [1,2]. *S. sclerotiorum*, which is the most common disease of oilseed rape (*Brassica napus* Linnaeus) in China [3], is dispersed through airborne ascospores or soilborne sclerotia. Airborne spores (ascospores, with a size

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https://doi.org/10.1016/j.heliyon.2024.e32429
Received 16 February 2024; Received in revised form 3 June 2024; Accepted 4 June 2024
Available online 6 June 2024

of 9–14 μm × 3–6 μm) are released during carpogenic germination from small mushroom-like structures called apothecia [4,5]. Exogenous nutrients such as senescent petals are required for ascospores to germinate and invade the host. Mycelium invasion is limited to enzymes or mechanical force, although some ascospores germinate into the stomata or wound as diffuse substances [6,7]. Petal infestation is a crucial stage in the cycle of SSR [8]. In addition, *S. sclerotiorum* can spread via insects, fieldwork or sap [9]. Dillard and Cobb [10] found that *S. sclerotiorum* cannot invade Brassicaceae without mechanical or insect-feeding damage. In addition, the range of the *S. sclerotiorum* infestation rates at feeding locations for lepidopteran larvae is 5%–60 %. Gao et al. [11] found that *S. sclerotiorum* can be transmitted by insects such as the honeybee *Apis cerana* (Fabricus) (Hymenoptera: Apidae) and the cabbage butterfly *Pieris rapae* (L.) (Lepidoptera: Pieridae) through experiments involving culturing their bodies and eluent, obtaining sclerotia, and re-culturing and inoculating the obtained sclerotia. Despite limited research, it appears that insects can promote *S. sclerotiorum* infestation.

Yamoah [12] proposed that insects could aid in fungal infection using three primary mechanisms: vectoring pathogen spores, creating wound sites for fungal penetration and establishing a conducive environment for fungal growth and development. Trichogramma wasps were found to possess a *Beauveria bassiana* (Bals.) Vuill. conidia carrying capacity of 10^4 orders of magnitude. The fluorescence of conidia can be observed throughout the body of insects, including the head, abdomen, wings and legs [13]. *Ectopsocus briggsi* (McLachlan) (Psocoptera: Ectopsocidae) spreads the conidia of *Spilocaea oleagina* (Castagne) Hughes, the causative agent of olive leaf spot, by carrying them on its body and acting as an internal vector [14]. Through feeding, egg-laying and habitat search, insects can cause various degrees of physical damage to plant tissues, which are often more susceptible to fungal infection than healthy tissues because wounds provide easy access for fungal invasion [12,15]. *Ceutorhynchidius troglodytes* (Fabricius) (Coleoptera: Curculionidae) can facilitate the spread of diseases by creating wounds that allow the pathogen *Phomopsis subordinaria* (Desmazières) to enter the plant host *Plantago lanceolata* (L.) (Plantaginaceae) [16]. In Europe, *Lobesia botrana* (Denis & Schiffermüller) (European grapevine moth; Lepidoptera: Tortricidae) significantly increases the severity of Botrytis bunch rot. Larvae carrying spores of *Botrytis cinerea* (Pers.: Fr) can infect grape berries by tunnelling through them to feed, creating wounds, and facilitating colonisation by spores on the berry surface [17–19]. Klein and Auld [20] pointed out that the damage caused by insects can result in plant cells breaking apart, which can release water and nutrients. They also found that *Colletotrichum orbiculare* (Berkeley and Montagne) infection in *Xanthium spinosum* (L.) (Compositae) can be more severe in plants damaged by insects. Insects may create a favourable environment for fungal infestation. Furthermore, both whitefly larvae and adults consume plant cell sap and secrete excess sugar onto leaves through honeydew. This substance can be used by the fungus *Metacapnodium fraserae* (S. Hughes) to form moulds on leaves and fruits [21], which reduces the photosynthetic capacity of the leaves and negatively affects plant growth [22].

Complex mutualistic processes may be involved when insects and fungal diseases coexist on a single plant. In China, aphids and *S. sclerotiorum* are the primary factors that reduce the yield of oilseed rape, and often co-occur on the same oilseed rape plant [3]. Aphids consume plant sap, which can result in stunted growth. They can also transmit various viruses, including those that affect the Brassicaceae family [23–28]. In addition, aphids excrete honeydew, which can lead to fungal growth [10]. However, the connection between aphids and *S. sclerotiorum* remains unknown.

Aphids are pests with piercing–sucking mouthparts. They create numerous wounds on the plant and secrete a small amount of gelatinous saliva on its surface because of their feeding behaviour [29]. During the insertion of the stylets into the intercellular spaces of the plant epidermis and mesophyll tissues, the sheath enveloping the stylets is formed by gelling saliva and quickly hardens to protect the stylets from mechanical damage and insulate the plant from chemical attack, as described by Tjallingii [29] and Tjallingii and Hogen-Esch [30]. When the stylet probes in the sieve tubes, an aphid secretes a large amount of water-soluble saliva into the phloem sap and begins to ingest the sap from the sieve tube. Water-soluble saliva is intermittently secreted by the aphid, which mixes with the sap in the sieve tube [29]. Water-soluble saliva has the primary function of inhibiting the plant's defence response to feeding [31]. In addition, aphids create favourable conditions for fungal infestation by secreting honeydew on plant leaves [31]. It appears that aphids have the prerequisites to encourage fungal invasion. *S. sclerotiorum* has had some facilitation in its occurrence in recent years as farmland rapeseed planting density has increased because of improved farming practices [7]. In particular, in some oilseed rape production areas, oilseed rape seedling stages have been showing an increase in *S. sclerotiorum* incidence, which overlaps with aphid incidence during the seedling stage. *S. sclerotiorum* incidence can be decreased by controlling aphids (personal communication). Although SSR has a higher chance of occurring in cool and wet conditions [6], spore dispersal is usually observed in warm and dry conditions with wind [32]. High temperatures, both locally and externally, and high humidity are favourable for aphids and *S. sclerotiorum* [33]. It is hypothesised that aphids play a crucial role in facilitating and complementing the transmission and infestation of *S. sclerotiorum*.

Is there a correlation between aphids and *S. sclerotiorum*? Does aphid feeding behaviour facilitate the spread of *S. sclerotiorum*? Is this relation influenced by oilseed rape cultivars? In the field, the greater the number of aphids on oilseed rape is, the more severe *S. sclerotiorum* is likely to occur. Thus, our investigation focused on the impact of aphid populations on *S. sclerotiorum* incidence in the field to explore the potential associations between aphids and *S. sclerotiorum*. In addition, we conducted an investigation to evaluate the effects of *S. sclerotiorum* on aphid feeding behaviour in two cultivars with different aphid resistance mechanisms in the lab using the electropenetrography (EPG) technique to understand the nature of aphid and *S. sclerotiorum* interactions and the effect of oilseed rape cultivars on this relation.

2. Materials and methods

2.1. Plants

Five cultivars of *Brassica napus* var. *napus* (L.) were chosen from the Laboratory of Plant Breeding of Anhui Academy of Agricultural Sciences, China: 'Deleyou6', 'Huashuang4', 'Huyou15', 'Zhongheza488' and 'Zhongshuang11'. 'Deleyou6' and 'Zhongshuang11' were used in all experiments in this study, with the remaining cultivars only used for determining the relation between *S. sclerotiorum* and aphids in the field.

The trial used sterilised pots and soil. Before planting, the oilseed rape seeds were subjected to surface sterilisation using 10 % sodium hypochlorite and 0.1 % Tween 20. The seeds were then washed with 96 % ethanol and rinsed five times with sterile distilled water according to the method reported by Boszoradova et al. [34]. Plants were cultivated in 13-cm-diameter plastic pots containing a mixture of peat moss, vermiculite, organic fertiliser ($N + P_2O_5 + K_2O$ 2 %, organic matter 40 %, Zhongnuo, Huaian, Jiangsu, China) and perlite (10:10:10:1 ratio). They were grown at a temperature of 25 °C ± 1 °C, 75 % ± 5 % relative humidity and a 12:12 (light: dark) photoperiod. They were regularly watered but did not receive additional fertiliser. Each *B. napus* cultivar was inoculated with either SSR or a mock inoculation when the plants had two fully expanded leaves. A 5-cm-diameter circle on a detached leaf of a four-week-old plant was spread with 1 g of mycelia (wet weight) and incubated in a sealed and humidified tray at room temperature [35]. These treated plants were used for aphid vectoring SSR and EPG studies during the four-leaf stage, following our previous research [36,37].

2.2. Aphids

Cabbage aphids, scientifically known as *Brevicoryne brassicae*, were acquired from oilseed rape plants cultivated in the greenhouse at the Institute of Vegetables, Zhejiang Academy of Agricultural Sciences. These aphids were bred in a controlled growth chamber for a period of 1 year on a *Brassica oleracea* var. *capitata* (L.) (Capparales: Brassicaceae) cultivar at a temperature of 25 °C ± 1 °C, 75 % ± 5 % relative humidity and a 16:8 (light:dark) photoperiod. All aphids used in this study were descendants of a single virginoparous apterous aphid. Individual aphids from this colony were used in the experiments detailed below.

2.3. Investigation of the aphid population in the greenhouse

According to the methodology reported by Hao et al. [38], a preference test was conducted to study the feeding behaviour of cabbage aphids. Throughout an oilseed rape growing season, which started on November 15, 2021, and ended on March 15, 2022, two oilseed rape cultivars, namely, 'Deleyou6' and 'Zhongshuang11', were evaluated in a greenhouse in Hefei (Anhui Province, 31°52′48″ N, 117°14′24″ E). A 4 m × 8 m plot was undivided and left open, with each 4 m × 4 m square being planted with a single cultivar in September (50-cm row spacing and 10-cm plant spacing). No chemical treatment was used, and the cultivation and management practices, including watering and fertilisation, mimicked those in the field. One hundred alate adult aphids were introduced to the central five plants of each cultivar at the two-leaf stage, and a monthly survey of the aphid population was performed by quantifying the number of nymphs and adults on the plants. Ten points were randomly selected for each cultivar, and at each point, three plants were randomly selected. All living aphids, both adults and nymphs, were counted on all branches of each plant.

2.4. Determination of host preference using a Y-tube olfactometer

The behavioural response of aphids to *B. napus* was determined using a series of Y-tube olfactometer behavioural bioassays, following the method reported by George et al. [38]. The odour source was provided by plants of two oilseed rape cultivars at the four-leaf stage, and clean air was used as a control. The Y-tube olfactometer consisted of colourless, transparent glass tubes with three arms, each measuring 10 cm in length and 2 cm in inner diameter. The angle between the two arms was 60°. Air was drawn through the activated carbon, odour source bottle and flow metre into both arms using the atmospheric collector as an air pump. The airflow rate was 100 mL/min, the room temperature was maintained at 20 °C ± 1 °C, the relative humidity was maintained at 70 % ± 5 %, and the light intensity was 3.25 μmol $m^{-2}s^{-1}$. One aphid was released at the halfway point of the glass tube. It was considered responsive when it reached an arm that was more than 5 cm away and vice versa. Each aphid was tested once, and the positions of the two arms were exchanged every five aphids. Each treatment was conducted using 30 aphids, and the number of responding aphids was recorded. At the end of the test, the Y-tube olfactometer and the link hose were wiped with anhydrous ethanol. All treatments were performed five times.

2.5. Fungi

Professor Li Qiangsheng from the Laboratory of Oilseed Rape Pests at Anhui Academy of Agricultural Sciences in China generously supplied a strain of *S. sclerotiorum*. The strain was selected for its virulence because it was collected from a location with notable disease prevalence. Cultures were prepared using techniques similar to those reported by Qandah [39], with minor adjustments.

S. sclerotiorum fungal cultures were cultivated in Difco potato dextrose broth (Becton, Dickinson and Company, New Jersey, USA) supplemented with 25 ppm ampicillin (MedChemExpress, Shanghai, China) and streptomycin (Sigma-Aldrich, Saint Louis, USA) in 200-mL Erlenmeyer flasks. The cultures were grown in darkness at room temperature (approximately 23 °C) with gentle agitation for

approximately 7 days until a large mass of mycelium had formed. The mycelium was washed with sterile water after decanting the supernatant [40]. Actively growing hyphae from these colonies were then transferred into dishes containing potato dextrose agar (PDA; Merck, Darmstadt, Germany) for sclerotia production. The PDA media were autoclaved for 20 min at 121 °C and 10^3 kPa. After the media had cooled, multiple 5-mm-diameter agar plugs containing hyphal tips from the isolate were inoculated into the dishes. The inoculated PDA media were sealed and incubated at 21 °C for 2 weeks with a 12-h light/dark cycle. After incubation, the sclerotia were removed from the surface of the PDA media. To condition all sclerotia for carpogenic germination, they underwent three successive cycles of 24 h of freezing at −20 °C followed by 24 h of thawing at 21 °C [39]. After the cycles, all sclerotia were disinfested by immersion in 5.76 % NaOCl for 1 min. They were then rinsed thrice with sterile distilled water (SDW) for 1 min each time and lightly blotted on sterilised filtre paper. Four disinfested sclerotia were half-buried in individual 9-cm-diameter polystyrene Petri dishes containing 10 g of sterile white sand. The sand in each dish was moistened by adding 4.1 mL of SDW until saturation. The dishes were then sealed with Parafilm and incubated in a growth chamber for 8 weeks, alternating between 12 °C/16 h of light and −18 °C/8 h of darkness. Germinated sclerotia with fully expanded apothecia were transferred to a new 5-cm dish containing a layer of water-saturated autoclaved white sand for ascospore collection. The lids were replaced every 1−2 days, and the ascospore-laden lids were stored in dry conditions at 4 °C until used. At the time of use, the ascospores were collected in vials by washing them off with 1 mL of SDW. Ascospore concentrations were estimated using a hemacytometer (Cambridge Scientific Instruments, Buffalo, USA) and adjusted to a suspension of 1×10^6 ascospores/mL. The suspension was then poured into a 50 mL plastic tube, following the methods reported by Qandah [39].

2.6. Identification of S. sclerotiorum

The confirmation of pathogen identification by Professor Fei Weixin, a plant pathologist, entails the validation of *S. sclerotiorum* by examining spore characteristics microscopically and cultivating spore-carrying aphids or diseased plant tissues on media to establish colonies.

2.7. Disease assessment

The resistance of the plants was assessed by inoculating of stems with inoculums, following the method reported by Mei et al. [41,42] and Zhu [43], with minor adjustments. The experiment took place in 2020–2021 using a completely randomised block design with two replications in a disease nursery plot situated in Hefei (Anhui Province, 31°52′48″ N, 117°14′24″ E), China. Twenty plants of each cultivar were planted in two rows, with 30-cm spacing between rows and 25 cm within rows. The two oilseed rape cultivars were sown on September 26. Field management followed standard breeding practices without the use of antifungal agents.

S. sclerotiorum was cultivated on PDA medium, comprising 20 % potato, 2 % dextrose, and 1.5 % agar. Sterilised toothpicks were arranged in a radial pattern in Petri dishes containing the PDA medium and co-cultured with the fungus in the dark at 20 °C. For each test line row, 10 plants were randomly selected and inoculated when 50 % of the plants in the row had at least one open flower [44]. The stems were pierced at a height of 20 cm above the ground using a 3-mm-diameter electric drill and then inserted with mycelium-covered toothpicks. Afterwards, the inoculated stems were wrapped with Parafilm® tape (Bemis Co, OshKosh, WI, USA) to maintain moisture. Plants were irrigated once daily for 10 min using overhead sprinklers for 3 days and then only when natural rainfall was insufficient for robust disease development. Stem lesion length was measured using a ruler 3 weeks after inoculation. Resistance was tested in 5–10 individuals from each plot. 'Zhongyou 821', a registered rapeseed cultivar in China known for its partial resistance against *S. sclerotiorum*, was used as a resistant control [45]. The susceptibility of the plants inoculated with *S. sclerotiorum* was measured 3 weeks after inoculation using lesion length on a scale of 0–4. A score of 0 indicated no visible symptoms, a score of 1 indicated a lesion length of 1–2 cm, a score of 2 indicated a lesion length of 2–3 cm, a score of 3 indicated a lesion length of 3–4 cm and a score of 4 indicated a lesion length of more than 4 cm. The resistance of oilseed rape to *S. sclerotiorum* was evaluated using the relative resistance index (RRI) (Table 1).

Disease index DI (%) = Σ[number of diseased plants at every scale × representative value at every scale]/[Total number of investigated plants × representative value at the highest scale] × 100.

Relative resistance index (RRI) = ln [DIm/(100 – DIm)] – ln [DIck/(100 – DIck)],

where:

RRI—relative resistance index of identified cultivars;

DIm—disease index of identified cultivars;

Table 1
Identification standard of oilseed rape resistance to *Sclerotinia sclerotiorum*.

Relative resistant index (RRI)	Evaluation of resistance
RRI ≤ −1.2	High resistance (HR)
−1.2< RRI ≤ −0.7	Medium resistance (MR)
−0.7 < RRI ≤0	Low resistance (LR)
0 < RRI ≤0.9	Low susceptibility (LS)
0.9 < RRI ≤2.0	Medium susceptibility (MS)
RRI >2.0	High susceptibility (HS)

DIck—disease index of the control cultivar 'Zhongyou 821'.

The disease index and relative resistance index were rounded to one decimal place [43].

2.8. Relation between aphid populations and S. sclerotiorum incidence in the field

Small-scale field plot experiments were conducted in three net houses (10 × 4.5 m/house) over a 3-year period (2019–2021) to assess two cultivars in Hefei (Anhui Province, 31°52′48″ N, 117°14′24″ E), China. The field sites had been used as Sclerotinia disease nurseries for several years to evaluate oilseed rape. The experiment consisted of field plots organised in a randomised block design, with each cultivar and pesticide treatment replicated thrice. Two pesticide treatments were administered: (a) control (no pesticide application) and (b) sprayed (pesticide application of Dingfeng® 0.27 kg/ha at seedling stage). The plots were sown and managed according to the recommended current farmer practices. Fertiliser was applied as required during the season to maintain high yields, but no fungicide was used. The aphids were placed on oilseed rape seedlings for approximately 5–7 days, after the establishment of the aphid population on the oilseed rape and the production of the next generation of oilseed rape aphids. During the flowering period of oilseed rape, the number of aphids was separately investigated in fields sprayed with insecticides and fields not sprayed with insecticides. In each field, 10 points were sampled in parallel lines, and 10 plants were continuously investigated in each point by rows [3]. SSR incidence in all experiments was recorded at the end of the growing season, approximately 1 week before harvest. One hundred randomly selected plants in each plot were assessed for disease presence. A plant was considered infected if the main stem or a branch was discoloured or shredded, with sclerotia present [46]. Disease incidence for each plot was calculated as the percentage of plants showing symptoms of SSR. Harvesting started approximately 30 days after final flowering, when 2/3 of the plant's pods were yellowish-green, the pods at the base of the main inflorescences turned loquat-yellow and the seed coat was blackish-brown. The oilseed rape seeds were weighed after drying [47].

2.9. Determination of the relation between aphids and S. sclerotiorum in a laboratory

2.9.1. Influence of aphid feeding on S. sclerotiorum incidence

Ten aphids were subjected to 24 h of starvation before being placed in a clip-cage (with bottom diameter × top opening diameter × depth of 30 × 40 × 35 mm), which was then fixed to the top leaf of the oilseed rape for 48 h. Following this period, the aphids were removed from the plant. Subsequently, four 1-mL droplets of ascospore suspension were carefully deposited on the leaf that had been fed on by the aphids using a micropipette. As a control, the plants were not subjected to aphid feeding. After inoculation, transparent domes were used to cover the trays containing the plants, thereby maintaining humidity levels above 85 %. The plants were then placed in the dark at a constant temperature of 22 °C ± 1 °C and 100 % relative humidity for 48 h. Subsequently, they were transferred to a greenhouse and incubated at a temperature of 18 °C−21 °C. Observations were conducted once a week for 4 weeks. To confirm that the observations made on the inoculated plants were a result of invasion by *S. sclerotiorum*, control plants were also inoculated without aphid feeding and were maintained under the same conditions as the inoculated plants [44]. These treatments were replicated five times.

2.9.2. Ability of aphids to carry ascospores of S. sclerotiorum

Ten cabbage aphids were introduced into cups containing a soft oilseed rape leaf. The stem was wrapped with moist cotton, and the insects were then sprayed with a spore suspension, following the method reported by Al-Shindah et al. [48]. These aphids were relocated and positioned on oilseed rape plants with four leaves in a transparent plastic cylindrical cage (16 cm in diameter and 28 cm in height) covered with a fine screen mesh (mesh size, 0.1 mm × 0.1 mm) to allow ventilation per plant. The cage was amintained in an incubator with a photoperiod (L:D) of 16:8 h at 22 °C ± 1 °C. After 72 h, the aphids were removed and the plants were subjected to darkness at 22 °C ± 1 °C and 100 % relative humidity for 2 days to observe the development of SSR. In addition, 10 other aphids or diseased plant tissues were directly cultured on media to form colonies for the confirmation of the presence of *S. sclerotiorum* by Prof. Fei Weixin. Ten aphids sprayed with sterile water were used as the control. Each treatment was replicated five times.

2.10. EPG experiments

The trial comprised three treatments for each oilseed rape cultivar: control (aphids without ascospores on healthy plants), treatment I (aphids without ascospores on infected plants) and treatment II (aphids with attached ascospores on healthy plants). All plants were at the four-leaf stage. Diseased plants and ascospore-carrying aphids were managed as described earlier. Aphid feeding behaviour was observed in each treatment using the EPG method developed by Tjallingii [49]. The methodology was as follows [36]: The dorsal abdomen of young aptera was gently attached to a gold wire (length 2 cm; diameter 18 μm) using a water-soluble electrically conductive silver glue (Electrolube, Swadlincote, Derbyshire, The United Kingdom). After tethering, aphids were placed back on the rearing plants overnight. At 0830 a.m. the next morning, each aphid was starved (aphids were placed in Petri dishes without food) for 1 h before the experiment [50]. After the aphid was transferred to a test plant, the wire electrode was connected to an amplifier. The aphid electrode was connected to a four-channel DC-EPG system (Giga-4; EPG Systems, Wageningen, The Netherlands), and the EPG output was recorded using PROBE 3.5 (hardware and software from EPG-Systems, Wageningen, The Netherlands). Inside a Faraday cage, the tethered aphid was quickly (<30 min after collecting from the rearing plant) placed on the upper surface of the mature leaf midrib of the target plant, which had a copper electrode inserted in the soil. Each aphid and plant were used just once for each recording. Successive recordings were for 6 h, according to our pre-experiment. Each treatment was documented more than 30 times in

a laboratory environment with consistent lighting and a temperature of 25 °C ± 1 °C. Each repetition consisted of one aphid and one plant, resulting in a total of 90 aphids and 90 plants. Abstract EPG waveforms were defined by correlation with stylet location in the plant and the intricate stylet activities performed therein, as shown in Table 2. The waveforms were grouped into distinguishable patterns, representing four main behavioural phases of functionally related activities (surface, epidermis–mesophyll, mesophyll and phloem phase). Waveforms were interpreted as follows: (i) non-probing (waveform np: stylets external to the plant), (ii) pathway phase (waveform C: including penetration, salivation, and other activities in the mesophyll plant tissues; waveform pd: [potential drop] meaning intracellular stylet punctures in the pathway phase) and (iii) phloem activities (waveform E: [E1 and E2] representing salivation into phloem sieve elements and phloem ingestion) [51]. EPG profiles were recorded using an A/D card (DI-710 format, Dataq Instruments Incorporated, The United States of America) and analysed using Stylet^{+} software. Data were automatically analysed using the MS Excel workbook for the automatic parameter calculation of EPG data (version 4.4) developed by Sarria et al. [52].

2.11. Statistical analyses

The data underwent transformations to satisfy the assumptions of normality of residuals and homoscedasticity for analysis of variance (ANOVA) analysis. The normality of the residuals was assessed using the Shapiro–Wilk's test, and homoscedasticity was assessed using Cochran's test for outlying variance. ANOVA with either Kruskal–Wallis test or Fisher's least significant difference was used for comparing different treatments and disease severity index, and significance was considered at $p \leq 0.05$ [44,53]. The EPG data were subjected to square-root transformation for the number of occurrences, natural log transformation for the duration and square-root arcsine transformation for the proportion. This study explored the relative acceptance of aphids and differences in responses to *S. sclerotiorum* infection between two oilseed rape cultivars. The differences in EPG variables between the control (feeding behaviour of aphids not carrying ascospores on uninfected plants), treatment I (feeding behaviour of aphids not carrying ascospores on infected plants) and treatment II (feeding behaviour of aphids with adherent ascospores on uninfected plants) on the two cultivars were compared. Non-Gaussian variables were analysed using the Mann–Whitney *U* test, and Gaussian variables were analysed using Student's *t*-test. Statistical analysis was performed using SAS 9.2 software (SAS Institute Inc., Cary, NC, USA). Two-way ANOVA was used to analyse the main effects of cultivar and infection status, as well as their interaction. A significance level of $p = 0.05$ was chosen [28]. Although all statistics were calculated for all variables, only the essential variables are presented in the tables and figures.

3. Results

3.1. Relation between aphid populations and S. sclerotiorum incidence in the field

The 3-year field trials revealed that insecticides had a significant impact on the decrease in the number of aphids but there was no significant effect on the thousand-seed weight. Aphid control reduced *S. sclerotiorum* incidence in the five cultivars, although this reduction was only evident in some cultivars (Table 3).

In 2019, the environment suppressed *S. sclerotiorum* incidence because of the dry and hot climate with major aphid outbreaks. However, in field tests, insecticide sprays significantly reduced aphid populations on oilseed rape plants, as well as *S. sclerotiorum* incidence, and aphid control significantly reduced *S. sclerotiorum* incidence in 'Huashuang4' ($p = 0.0322$) and 'Huyou15' ($p = 0.0368$). The thousand-seed weight was not significantly affected by aphid control. In 2020, in five cultivars, insecticide spraying provided good

Table 2
The definitions of the waveforms scored in the electropenetrography (EPG) analyses.

Acronym[a]	Variable type (Unit)	Definition
Surface-mesophyll (Leaf)		
t_1Pr	Time(s)	Time to the first probe from the start of EPG
n_bPr	Frequency	Number of short probes (C < 3 min)
t_1C.1pd	Time(s)	Time from the beginning of the 1st probe to the first pd
n_pd	Frequency	Number of pd
s_pd	Time(s)	Total duration of pd
t_1EinPr	Time(s)	Time from the beginning of that probe to the 1st E
s_C	Time(s)	Total C duration with pd
%probtimeinC	Index (%)	% of probing spent in C
Phloem		
n_E1	Frequency	Number of E1 periods
s_E1	Time(s)	Total duration of E1
d_E1followedby1sE2	Time(s)	Duration of the E1 followed by the first sustained E2 (>10 min)
%_E1/E12	Index (%)	Relative amount of E1 on E12
s_E2	Time(s)	Total duration of E2 periods
s_longestE2	Time(s)	Duration of the longest E2
E2index	Index (%)	phloemian index: % of the time of the E2 after the start of the 1st E2
%sE2/E2	Index (%)	Relative amount of sE2 on E2
%probtimeinE1	Index (%)	% of probing spent in E1
%probtimeinE2	Index (%)	% of probing spent in E2

[a] All of the variables were analysed within 6 h.

Table 3
Relation between aphid infestation and *Sclerotinia sclerotiorum* incidence in the field.

Cultivar[a]	Mean total number of aphids/100 plants	Mean total incidence of *S. sclerotiorum* %/100 plants	Mean total thousand-seed weight (g)	Mean total number of aphids after insecticide application/100 plants	Mean total incidence of *S. sclerotiorum* following insecticide application %/100 plants	Mean total thousand-seed weight after insecticide application (g)
2019						
Deleyou6	16438.50 ± 2773.55*	14.69 ± 4.42	4.32 ± 0.28	870.29 ± 73.90	4.74 ± 0.88	4.82 ± 0.23
Huashuang4	11643.77 ± 1596.60*	7.60 ± 1.12*	4.01 ± 0.21	470.29 ± 83.14	2.84 ± 0.97	4.25 ± 0.26
Huyou15	10220.04 ± 1069.28*	5.65 ± 1.09*	3.73 ± 0.22	433.19 ± 57.45	1.33 ± 0.88	4.57 ± 0.26
Zhongheza488	7187.40 ± 908.14*	2.75 ± 1.15	3.73 ± 0.24	241.06 ± 46.45	1.98 ± 0.61	3.72 ± 0.13
Zhongshuang11	5894.40 ± 650.75*	1.90 ± 0.49	3.91 ± 0.06	218.97 ± 63.37	1.27 ± 0.64	3.97 ± 0.27
2020						
Deleyou6	3996.67 ± 449.79*	42.15 ± 3.66	3.05 ± 0.13	14.76 ± 3.83	34.39 ± 4.15	3.11 ± 0.07
Huashuang4	3168.10 ± 144.43*	37.74 ± 4.99	3.06 ± 0.24	13.81 ± 1.32	31.71 ± 4.60	3.10 ± 0.23
Huyou15	4035.24 ± 138.96*	55.65 ± 1.77	3.65 ± 0.30	10.48 ± 1.44	43.21 ± 4.44	3.77 ± 0.28
Zhongheza488	4823.81 ± 333.02*	45.84 ± 4.08	3.23 ± 0.18	6.19 ± 1.91	37.37 ± 2.24	3.24 ± 0.14
Zhongshuang11	4012.38 ± 92.75*	41.17 ± 5.35*	3.78 ± 0.19	8.57 ± 3.30	20.86 ± 3.66	3.73 ± 0.24
2021						
Deleyou6	5220.00 ± 647.36*	53.50 ± 3.84	3.23 ± 0.18	11.43 ± 2.38	40.16 ± 3.90	3.18 ± 0.10
Huashuang4	1700.48 ± 173.62*	22.25 ± 1.91*	3.92 ± 0.17	9.52 ± 2.02	13.24 ± 2.31	4.10 ± 0.35
Huyou15	1719.05 ± 202.57*	27.98 ± 3.23	3.57 ± 0.26	6.67 ± 1.20	21.33 ± 1.45	3.60 ± 0.26
Zhongheza488	3950.00 ± 224.86*	35.37 ± 2.38	3.29 ± 0.15	5.71 ± 1.65	28.16 ± 1.30	3.26 ± 0.14
Zhongshuang11	5905.71 ± 197.73*	48.59 ± 2.35*	3.56 ± 0.11	3.33 ± 0.88	11.58 ± 2.10	3.75 ± 0.22

[a] Values are means ± SEM. Data were analysed using Student's *t*-test. The significance level was set at $p < 0.05$. The asterisk after a value represents a significant difference in variables between the treatments with and without aphid control for each cultivar. Means of aphids after insecticide application/100 plants mean that the data refer to the plots where insecticides were applied. Means of aphids/100 plants refers to the plots without insecticide treatment.

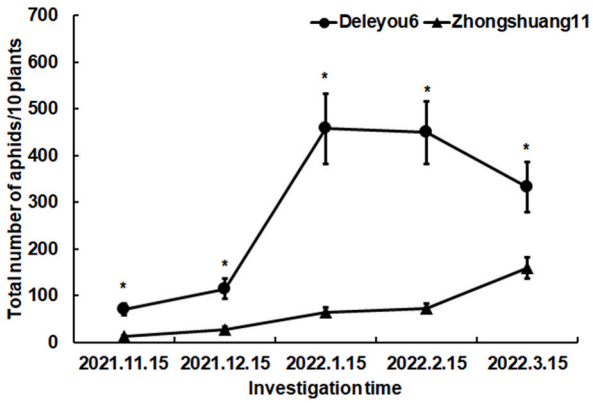

Fig. 1. Dynamics of cabbage aphids on two oilseed rape cultivars in the field. Values are means ± SEM of the total number of aphids (adults and nymphs)/10 plants. Data were analysed using Student's *t*-test. The significance level was set at $p < 0.05$. Bars represent standard error (SE). * represents significant differences between the two cultivars at the same investigation time. All living aphids, both adults and nymphs, were counted on all branches of each plant.

control of aphids. For each cultivar, the reduction in the aphid population and *S. sclerotiorum* incidence were suppressed by the use of insecticides, particularly in 'Zhongshuang11', where *S. sclerotiorum* incidence was significantly reduced to approximately 21 % after the reduction of aphid population ($p = 0.0351$). The thousand-seed weight was not affected by insecticide application. In 2021, spraying insecticides could effectively control aphids in five oilseed rape cultivars. With the reduction in aphid population, *S. sclerotiorum* incidence was also reduced in all cultivars, particularly in 'Huashuang4', where the reduction in *S. sclerotiorum* incidence reached a significant level ($p = 0.0398$). The thousand-seed weight was not affected by the application of insecticides. Correlation analysis also showed that in 2019, the number of aphids was significantly correlated with *S. sclerotiorum* incidence with a correlation coefficient of 0.8481 ($p = 0.0019$), and the correlation between the number of aphids and the thousand-seed weight and that between *S. sclerotiorum* incidence and the thousand-seed weight were not significant. A strong positive correlation was maintained between the aphid population and *S. sclerotiorum* incidence in 2020–2021, i.e. an increase in aphid number was accompanied by an increase in *S. sclerotiorum* incidence and vice versa. Negative but insignificant correlations were observed between the aphid population and the thousand-seed weight and between *S. sclerotiorum* incidence and the thousand-seed weight (Table 3).

3.2. Identification of aphid resistance in two cultivars

The aphid population on the two winter oilseed rape cultivars was surveyed on a fixed date every month between November 2021 and March 2022. The results showed that the aphid population on 'Deleyou6' reached a peak of 457.50 ± 75.83 in January 2022 and then gradually decreased, whereas the aphid population on 'Zhongshuang11' gradually increased and reached a peak of 159.82 ± 22.28 in March 2022. Moreover, the number of aphids on 'Deleyou6' was significantly higher than that on 'Zhongshuang11' at each time point of the survey (Fig. 1).

3.3. Host selection studies

As shown in Table 4, the attractiveness of 'Deleyou6' to aphids was significantly higher than that of 'Zhongshuang11'. 'Deleyou6' attracted 94 % of aphids compared with 63 % of aphids on 'Zhongshuang11'.

3.4. Sclerotinia disease assessment

Artificial inoculation with *S. sclerotiorum* was used to determine each oilseed rape cultivar's resistance or susceptibility to the fungus according to the criteria for resistance level classification.

Table 5 shows the RRI for three replications. The average RRI of 'Deleyou6' in three assessments was −0.89, indicating medium resistance to *S. sclerotiorum*. By contrast, the average value of 'Zhongshuang11' in three tests was −0.82, indicating medium resistance to *S. sclerotiorum*.

3.5. Influence of aphid behaviour on S. sclerotiorum incidence

3.5.1. Influence of aphid feeding and non-feeding on S. sclerotiorum incidence

Significant differences in *S. sclerotiorum* incidence in both cultivars after both aphid non-feeding and post-feeding plants were found. In 'Deleyou6', *S. sclerotiorum* incidence increased from 26 % to 74 % after aphid feeding ($F = 4.53$, $p = 0.0010$). Similarly, in 'Zhongshuang11', *S. sclerotiorum* incidence increased threefold to 72 % after aphid feeding compared with 24 % on plants that had not been fed on by aphids ($F = 1.29$, $p = 0.0005$). *S. sclerotiorum* incidence was not significantly different between the two cultivars, regardless of feeding ($F = 1.89$, $p = 0.7173$ for not feeding; $F = 1.85$, $p = 0.7957$ for feeding) (see Fig. 2).

3.5.2. Ability of aphids to transmit ascospores

When aphids are sprayed with ascospores and then introduced into healthy oilseed rape plants, they can lead to *S. sclerotiorum* infection. Infection incidence in both 'Deleyou6' and 'Zhongshuang11' can reach over 10 %, with no significant difference between the two cultivars (refer to Table 6).

3.6. Determination of aphid feeding behaviour using the EPG technique

3.6.1. Treatment I—changes in the feeding behaviour of aphids in plants infected with S. sclerotiorum

There was a significant difference in the time for aphids to start the first puncture (t_1Pr) in the two cultivars, which was significantly shorter in 'Deleyou6' than in 'Zhongshuang11' ($U = 59$, $p = 0.0001$). The time for aphids to start probing was significantly

Table 4
Host selectivity of aphids measured using a Y-tube olfactometer.

Cultivar[a]	Attractant rate	Standard error	*F*	*df*	*t*	*P*
Deleyou6	0.94	0.04	1.00	8	5.67	0.0005
Zhongshuang11	0.63	0.04				

[a] Values are means ± SEM. Data were analysed using Student's *t*-test after arcsine transformations. The significance level was set at $p < 0.05$.

Table 5
Artificial inoculation results of *Sclerotinia sclerotiorum*.

Cultivar	Relative resistant index (RRI)			Evaluation of resistance
	1	2	3	
Deleyou6	−0.93	−0.84	−0.91	MR
Zhongshuang11	−0.73	−0.91	−0.81	MR

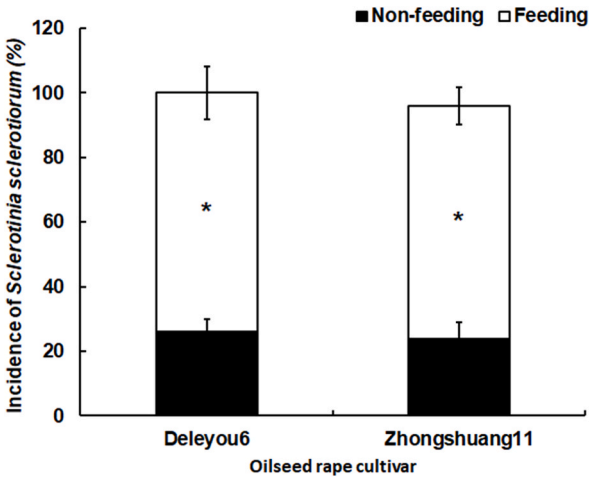

Fig. 2. Influence of aphid feeding on *Sclerotinia sclerotiorum* incidence in the two oilseed rape cultivars 'Deleyou6' and 'Zhongshuang11'. The values in the figure are percentages of infected plants and represent mean ± standard error (SE). The black columns represent *S. sclerotiorum* incidence in plants that were not fed by aphids, and the white columns represent *S. sclerotiorum* incidence in plants that were fed by aphids. The asterisk in the middle of the white columns indicates a statistical difference between unfed and fed plants of the same cultivar.

Table 6
Ability of aphid transmitting *Sclerotinia sclerotiorum*.

Cultivar[a]	Incidence of *Sclerotinia sclerotiorum*	Standard error	*F*	*df*	*t*	*P*
Deleyou6	0.14	0.05	1.86	8	0.32	0.7599
Zhongshuang11	0.12	0.04				

[a] Values are means ± SEM. Data were analysed using Student's *t*-test after arcsine transformations. The significance level was set at $p < 0.05$.

shorter in both cultivars after plants were infected with *S. sclerotiorum*, i.e. from 142 to 89 s in 'Deleyou6' ($U = 82$, $p = 0.0015$) and from 403 to 130 s in 'Zhongshuang11' ($U = 44$, $p < 0.0001$) (Fig. 3A).

In the mesophyll, the frequency of the brief probes (n_bPr) of aphids was significantly lower in 'Deleyou6' (1.8) than in 'Zhongshuang11' (3.3). After *S. sclerotiorum* infection of the plants, aphids significantly increased the frequency of brief probes in both 'Deleyou6' and 'Zhongshuang11', with a nearly ninefold increase in 'Deleyou6' ($U = 42$, $p < 0.0001$) (Fig. 3B). Following plant infection with *S. sclerotiorum*, aphids significantly increased the frequency of intracellular punctures (n_pd) to 155 ($t = -4.43$, $p = 0.0001$) in infected 'Deleyou6' and 132 ($t = -2.41$, $p = 0.0208$) in infected 'Zhongshuang11' (Fig. 3C). The time for aphids to puncture mesophyll cell (s_pd) was significantly shorter in 'Deleyou6' than 842 s in 'Zhongshuang11'. However, after the plants were infected with *S. sclerotiorum*, this time was significantly increased to 646 s in 'Deleyou6' ($t = -2.65$, $p = 0.0117$), whereas there was no significant change in 'Zhongshuang11' (Fig. 3D). The time between the start of the first probe and the first intracellular puncture (t_1C.1pd) was not significantly different between the two cultivars, but after *S. sclerotiorum* infection of the plants, this time was significantly shortened to approximately 146 s in both 'Deleyou6' and 'Zhongshuang11' (Fig. 3E). The time that aphids spent between the start of that probe and before the first phloem contact (t_1EinPr) was not significantly different between the two cultivars, but the time that aphids spent before the first phloem contact was shortened from more than 3000 s to more than 1000 s after the *S. sclerotiorum* infection in plants (Fig. 3F). The time spent by aphids in the pathway period (s_C) was 12,325 s in 'Deleyou6', which was significantly shorter than 17,708 s in 'Zhongshuang11', and after *S. sclerotiorum* infection of the plants, aphids significantly shortened the time in the pathway period only in 'Zhongshuang11' ($U = 45$, $p < 0.0001$) (Fig. 3G). The percentage of time spent in the pathway period (%probtimeinC) was 52.61 % in 'Deleyou6', significantly lower than 76.27 % in 'Zhongshuang11'. This percentage did not change significantly in 'Deleyou6' after *S. sclerotiorum* infection of the plants, but in 'Zhongshuang11', it was significantly decreased ($U = 40$, $p < 0.0001$) (Fig. 3H).

The frequency of aphid salivary secretion (n_E1) did not differ significantly between the two cultivars, and showed a significant

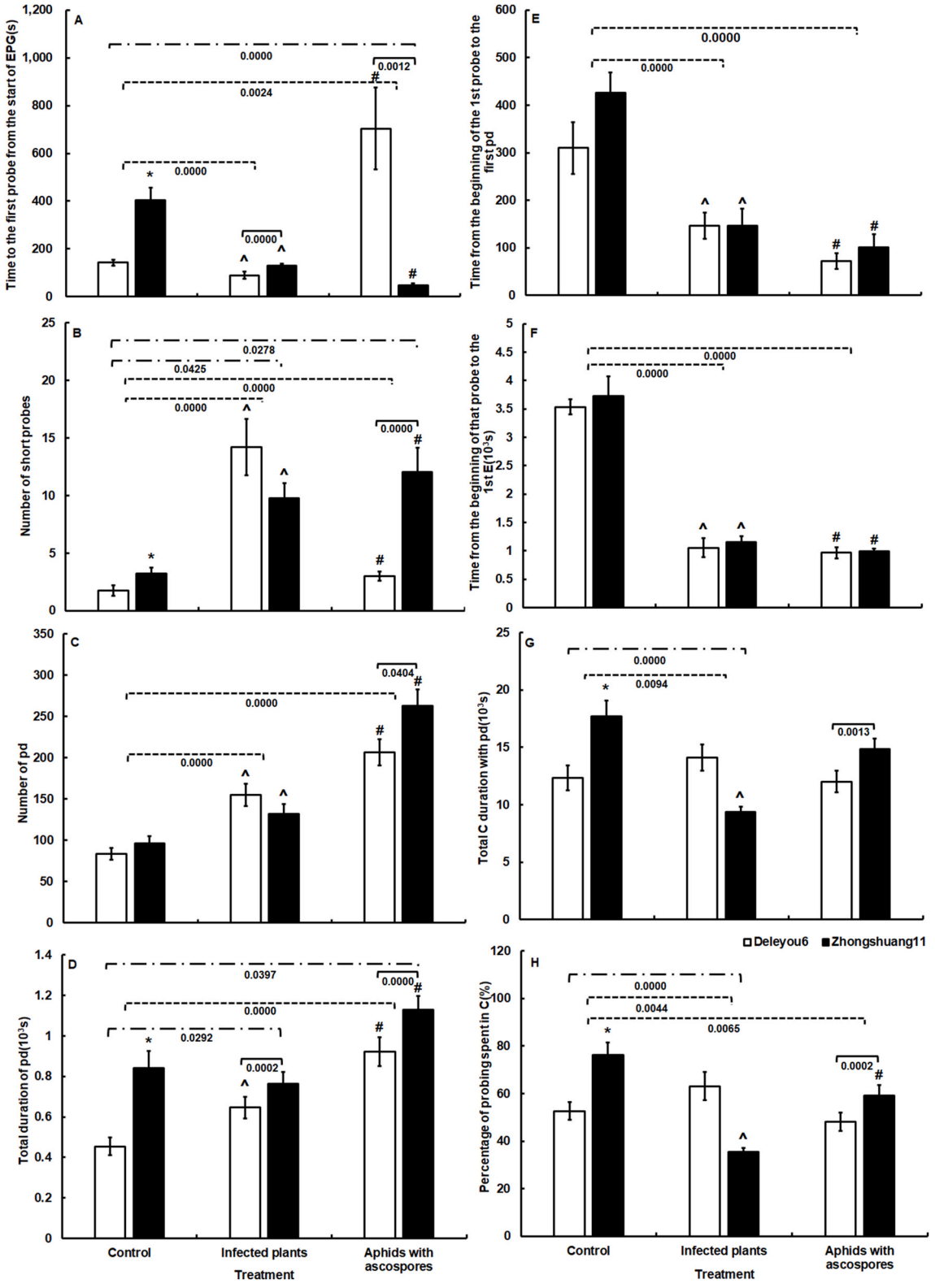

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Fig. 3. The major surface-mesophyll-related variables of *Brevicoryne brassicae* probing behaviour in two oilseed rape cultivars in *Sclerotinia sclerotiorum*-infected and uninfected treatments. The figure's values display mean ± standard error (SE). Following the square-root transformation for frequency variables, natural log transformation for time variables and square-arcsine for percentage variables, these data were compared using Student's *t*-tests (for Gaussian variables) or Mann–Whitney *U*-tests (for non-Gaussian variables). The significance level was set at $p < 0.05$. * on the columns denotes a statistical difference between the controls of the two oilseed rape cultivars. ˆ on the columns indicates a statistical difference between infected plant and control in the same cultivar. # on the columns indicates a statistical difference between aphids with ascospores and the control in the same cultivar. (A) Time to the first probe from the start of EPG, (B) number of short probes, (C) number of pd, (D) total duration of pd, (E) time from the beginning of the first probe to the first pd, (F) time from the beginning of that probe to the first E, (G) total C duration with pd, (H) percentage of probing spent in C. Two-way ANOVA was used to analyse the main effects of cultivar and infection status, as well as their interaction. '——' represents the cultivar main effects on the infection or uninfection of *S. sclerotiorum* in plants, or on whether aphids carry ascospores and not, and the numbers below represent *p*-values. '———' represents the main effects of *S. sclerotiorum* infection on the infection or uninfection of *S. sclerotiorum* in plants, or on whether aphids carry ascospores and not, and the numbers below represent *p*-values. '– - – -' represents the main effects of the cultivar and *S. sclerotiorum* infection interaction on the infection or uninfection of *S. sclerotiorum* in plants, or on whether aphids carry ascospores and not, and the numbers below represent *p*-values.

increase only in 'Deleyou6' to 4.4 times ($U = 104$, $p = 0.0099$) after the plants were infected with *S. sclerotiorum* (Fig. 4A). The total duration of aphid salivary secretion (s_E1) was only 89 s in 'Deleyou6' and up to 315 s in 'Zhongshuang11' ($U = 10$, $p < 0.0001$). After plants were infected with *S. sclerotiorum*, aphids significantly increased the duration of salivary secretion in 'Deleyou6' ($U = 84$, $p = 0.0018$), but this duration was significantly shorter in 'Zhongshuang11' than in the controls ($U = 76$, $p = 0.0008$) (Fig. 4B). In the phloem of the infected plants, the aphid salivary secretion time before the first sustained feeding (d_E1followedby1sE2) was significantly shorter in both cultivars than in the controls, i.e. 28 s in 'Deleyou6' ($t = 13.35$, $p < 0.0001$) and 51 s in 'Zhongshuang11' ($U = 47$, $p < 0.0001$) (Fig. 4C). The percentage of salivary secretion by aphids in the phloem stage in 'Deleyou6' was significantly lower (0.81 %) than in 'Zhongshuang11' (29.39 %) ($U = 26$, $p < 0.0001$). After plants were infected with *S. sclerotiorum*, aphids significantly increased this percentage in 'Deleyou6' to 2.52 % ($U = 30$, $p < 0.0001$) whereas it was significantly decreased in 'Zhongshuang11' to 1.53 % ($U = 46$, $p < 0.0001$) (Fig. 4D). The proportion of aphids secreting saliva to total probing duration (%probtimeinE1) was significantly lower in 'Deleyou6' than in 'Zhongshuang11' ($U = 81$, $p = 0.0013$). The proportion did not change significantly in 'Deleyou6' after plants were infected with *S. sclerotiorum*, but it significantly decreased to 0.45 % in 'Zhongshuang11' ($t = 2.49$, $p = 0.0174$) (Fig. 4E). The duration of aphid feeding in the phloem (s_E2) was 13,141 s in 'Deleyou6', which was significantly longer than 7412 s in 'Zhongshuang11' ($U = 76$, $p = 0.0008$), and the duration of feeding was significantly shortened to 10,244 s in 'Deleyou6' only after the plants were infected with *S. sclerotiorum* ($t = 2.24$, $p = 0.0313$) (Fig. 4F). The index of feeding (E2index) was significantly higher in 'Deleyou6' than in 'Zhongshuang11' ($U = 87$, $p = 0.0024$). After plants were infected with *S. sclerotiorum*, the feeding index was significantly lower only in 'Deleyou6' ($t = 9.90$, $p < 0.0001$) (Fig. 4H). In the phloem, the percentage of sustained ingestion by aphids (%sE2/E2) was significantly higher in 'Deleyou6' than in 'Zhongshuang11' ($U = 62.5$, $p = 0.0002$). After plants were infected with *S. sclerotiorum*, the percentage of sustained ingestion by aphids in diseased plants was significantly lower in 'Deleyou6' ($U = 69.5$, $p = 0.0004$) and significantly higher in 'Zhongshuang11' to 76.33 % ($t = -2.36$, $p = 0.0233$) than in the controls (Fig. 4I). The percentage of aphids used in the feeding stage (%probtimeinE2) was also significantly higher in 'Deleyou6' (47.22 %) than in 'Zhongshuang11' (21.79 %), and this percentage was significantly lower on 'Deleyou6' only after *S. sclerotiorum* infection ($t = 3.91$, $p = 0.0004$) (Fig. 4J). The aphids spent a significantly longer maximum feeding period (s_longestE2) in 'Deleyou6' than in 'Zhongshuang11' ($U = 80$, $p = 0.0012$). After plants of 'Deleyou6' were infected with *S. sclerotiorum*, the longest feeding period spent by aphids was shortened to 4105 s compared with 11,906 s in the control ($t = 12.37$, $p < 0.0001$) and there was no significant change in 'Zhongshuang11' (Fig. 4G).

3.6.2. Treatment II—the effect of ascospores on the feeding behaviour of aphids

Compared with aphids not carrying ascospores, aphids carrying ascospores significantly delayed the time to the first start of penetration to 704 s in 'Deleyou6' ($U = 117$, $p = 0.0257$) and significantly shortened the time to 48 s in 'Zhongshuang11' ($t = 8.88$, $p < 0.0001$) (Fig. 3A).

Aphids carrying ascospores showed a significant increase in the frequency of brief probes compared with aphids not carrying ascospores, increasing to 12 in 'Zhongshuang11' ($U = 70.5$, $p = 0.0005$) (Fig. 3B). Aphids carrying ascospores significantly increased the frequency of intracellular punctures compared with aphids not carrying ascospores in both 'Deleyou6' and 'Zhongshuang11' (Fig. 3C) and significantly increased the duration of intracellular punctures to approximately 1000 s in both cultivars (Fig. 3D). Aphids carrying ascospores significantly shortened the time from the start of the first penetration to the first intracellular puncture in 'Deleyou6' and 'Zhongshuang11' compared with aphids not carrying ascospores (Fig. 3E). The time from the start of the probe to the first phloem contact was significantly shorter in aphids carrying ascospores than in aphids not carrying ascospores, with a reduction of more than 900 s in both cultivars (Fig. 3F). Compared with aphids not carrying ascospores, the percentage of time spent in the pathway period by aphids carrying ascospores did not change significantly in 'Deleyou6', but this percentage was significantly lower in 'Zhongshuang11' ($t = 2.79$, $p = 0.0082$) (Fig. 3H).

Aphids carrying ascospores showed a significant increase in the frequency of salivary secretion over aphids not carrying ascospores, increasing to 10.1 times in 'Deleyou6' ($t = -12.39$, $p < 0.0001$) and 9.8 times in 'Zhongshuang11' ($t = -7.00$, $p < 0.0001$) (Fig. 4A). The total duration of salivary secretion was significantly longer in aphids carrying ascospores than in controls in both cultivars (Fig. 4B). However, the salivary secretion time before the first sustained feeding was significantly reduced in aphids carrying ascospores than in aphids not carrying ascospores (Fig. 4C). The percentage of salivary secretion within the phloem stage in aphids carrying

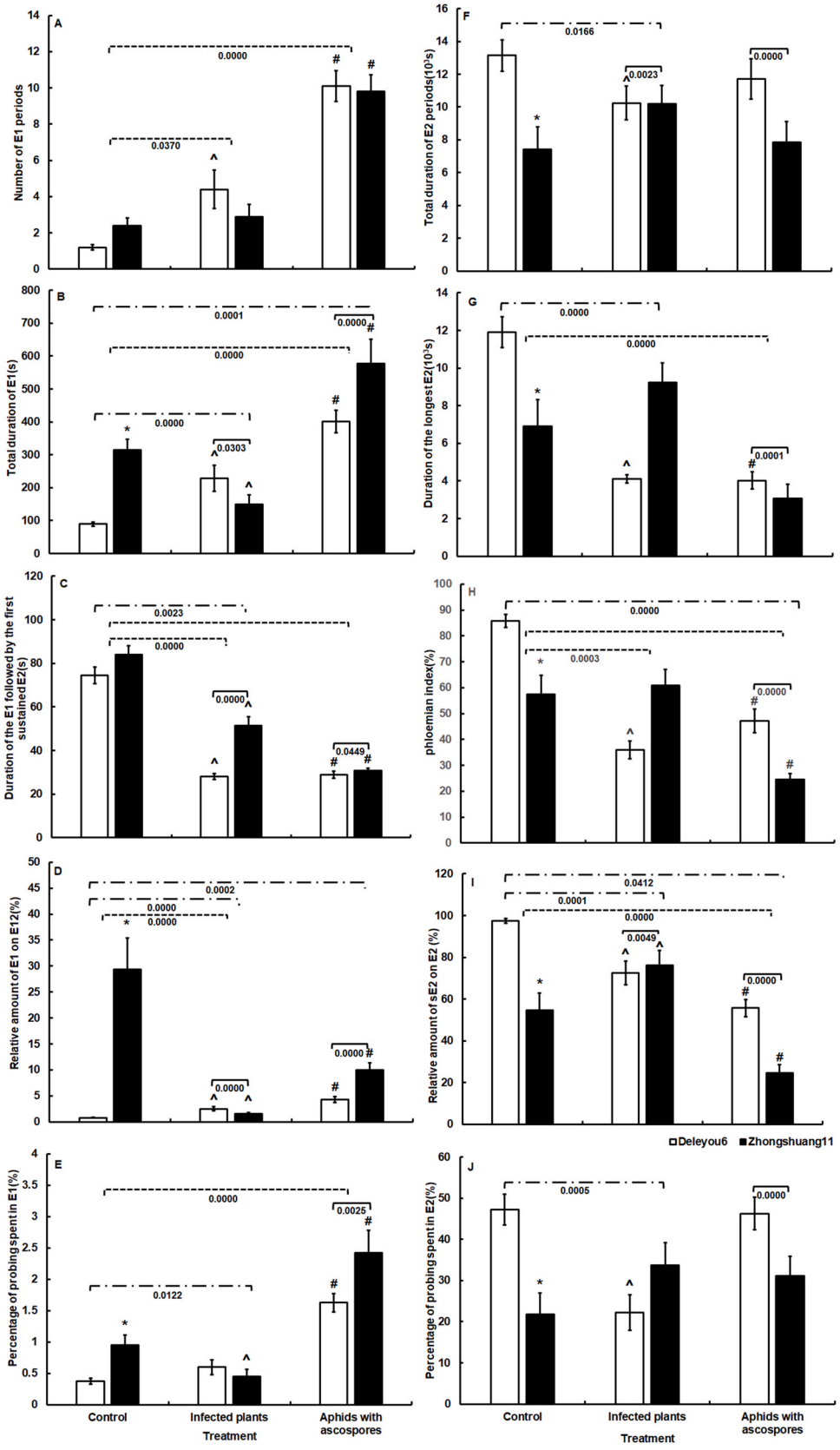

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Fig. 4. The major phloem-related variables of *Brevicoryne brassicae* probing behaviour in two oilseed rape cultivars in *Sclerotinia sclerotiorum*-infected and uninfected treatments. The figure's values display mean ± standard error (SE). Following the square-root transformation for frequency variables, natural log transformation for time variables and square-arcsine for percentage variables, these data were compared using Student's *t*-tests (for Gaussian variables) or Mann–Whitney *U*-tests (for non-Gaussian variables). The significance level was set at $p < 0.05$. * on the columns denotes a statistical difference between the controls of the two oilseed rape cultivars. ^ on the columns indicates a statistical difference between infected plant and control in the same cultivar. # on the columns indicates a statistical difference between aphids with ascospores and control in the same cultivar. (A) Number of E1 periods, (B) total duration of E1, (C) duration of the E1 followed by the first sustained E2, (D) relative amount of E1 on E12, (E) percentage of probing spent in E1, (F) total duration of E2 periods, (G) duration of the longest E2, (H) percentage of the time of the E2 after the start of the first E2 (Phloemian index), (I) relative amount of sE2 on E2, (J) percentage of probing spent in E2. Two-way ANOVA was used to analyse the main effects of cultivar and infection status, as well as their interaction. '——' represents the cultivar main effects on the infection or uninfection of *S. sclerotiorum* in plants, or on whether aphids carry ascospores and not, and the numbers below represent *p*-values. '———' represents the main effects of *S. sclerotiorum* infection on the infection or uninfection of *S. sclerotiorum* in plants, or on whether aphids carry ascospores and not, and the numbers below represent *p*-values. '– - – -' represents the main effects of the cultivar and *S. sclerotiorum* infection interaction on the infection or uninfection of *S. sclerotiorum* in plants, or on whether aphids carry ascospores and not, and the numbers below represent *p*-values.

ascospores compared with the control was significantly increased to 4.33 % in 'Deleyou6' ($U = 19$, $p < 0.0001$) and significantly decreased to 10.01 % in 'Zhongshuang11' ($U = 116$, $p = 0.0238$) (Fig. 4D). The percentage of salivary secretion of aphids carrying ascospores was significantly increased to 1.62 % in 'Deleyou6' ($t = -9.06$, $p < 0.0001$) and to 2.42 % on 'Zhongshuang11' ($t = -3.86$, $p = 0.0004$) compared with the control (Fig. 4E). The feeding index of aphids carrying ascospores was significantly reduced in both cultivars than in aphids not carrying ascospores (Fig. 4H). In the phloem of 'Deleyou6', the percentage of sustained ingestion (%sE2/E2) in aphids carrying ascospores was only 55.69 %, which was significantly lower than that of the control at 97.44 % ($t = 10.42$, $p < 0.0001$). The percentage of aphids carrying ascospores that sustained ingestion on the phloem of 'Zhongshuang11' was also significantly lower than that of the control ($U = 112$, $p = 0.0178$) (Fig. 4I). Aphids carrying ascospores significantly shortened the duration of the longest feeding period in 'Deleyou6' compared with the control ($U = 6$, $p < 0.0001$), and there was no significant change in 'Zhongshuang11' (Fig. 4G).

3.6.3. Analysis of cultivar and S. sclerotiorum infection main effects

The time at which aphids began their first leaf probing after *S. sclerotiorum* infection of oilseed rape plants was significantly affected by the cultivar and *S. sclerotiorum* infestation but not by the cross-interaction between the two. *S. sclerotiorum* infestation and the interplay between cultivars and *S. sclerotiorum* infestation contributed to the influence of aphids on the number of brief probes in infected mesophyll. *S. sclerotiorum* infestation had a major impact on both the timing of aphids' initial cell puncture and the frequency of intracellular punctures. By contrast, the cultivar and the relation between the cultivar and *S. sclerotiorum* infestation affected the duration of intracellular punctures. The infestation of *S. sclerotiorum* was the main factor affecting the pathway duration (Fig. 3).

In the phloem of infected plants, the frequency of saliva secretion by aphids was only affected by the *S. sclerotiorum* infestation and the duration of saliva secretion was affected by the cultivar and the cross-interaction between the cultivar and *S. sclerotiorum* infestation. The cultivar, *S. sclerotiorum* infestation and the cross-interaction between them affected saliva secretion before ingestion and the percentage of saliva secretion in the phloem. Ingestion was mainly influenced by the cultivar and the interactions between the cultivar and *S. sclerotiorum* infestation, and the phloemian index was influenced by the *S. sclerotiorum* infestation and the interactions between the cultivar and *S. sclerotiorum* infestation.

The time to start the first leaf surface puncture after the aphid carried ascospores was influenced by the cultivar, *S. sclerotiorum* infestation and cross-interaction between the two. In the mesophyll, the time at which aphids carrying ascospores began their first cellular puncture was affected only by *S. sclerotiorum* infestation and the frequency of brief probes was influenced by the cultivar, *S. sclerotiorum* infestation and the interactions between the cultivar and *S. sclerotiorum* infestation. The cultivar and *S. sclerotiorum* infestation had a significant impact on the frequency and duration of intracellular punctures. Conversely, the duration of the pathway phase was influenced only by the cultivar, and the percentage of the pathway phase was influenced by both the cultivar and *S. sclerotiorum* infestation (Fig. 3).

As shown in Fig. 4, the frequency of salivary secretion of aphids carrying ascospores was mainly affected by ascospores adhering to the aphids. The total duration of salivary secretion of aphids carrying ascospores was influenced by the cultivar, ascospore adhesion and cross-interaction between the two. Saliva secretion before feeding was affected by the cultivar and *S. sclerotiorum* infestation, and the percentage of salivary secretion in the phloem was affected by the cultivar and the interaction between the cultivar and *S. sclerotiorum* infestation. The cultivar and *S. sclerotiorum* infestation affected the variable, i.e. percentage of total salivary secretion (% probtimeinE1). The cultivar was the primary factor in ingestion, and some variables related to it, such as s_longestE2, E2index, and % sE2/E2, were also affected by *S. sclerotiorum* infestation.

4. Discussion

A strong association between *S. sclerotiorum* and aphids in oilseed rape has be found. The winter oilseed rapes 'Deleyou6', 'Huashuang4', 'Huyou15', 'Zhongheza488' and 'Zhongshuang11' showed a correlation between higher aphid population and increased occurrence of *S. sclerotiorum*. Controlling the aphid population with insecticides decreased *S. sclerotiorum* incidence. The correlation analysis of the data from the 3 years of study revealed that the significance values of the correlation coefficients between aphid numbers and *S. sclerotiorum* incidence were all less than 0.05, indicating a significant correlation, which also suggests that there is a

correlation between aphids and *S. sclerotiorum*. The relation between insects and fungi can be mutualism [54–56], commensalism and antagonism [57,58]. These interactions can have essential effects on the behaviour, reproductive success, population dynamics and evolution of both insects and their fungal associates [59]. The type of relation that exists between aphids and *S. sclerotiorum* is not clear.

The results of host selection in this study showed that 'Deleyou6' was more attractive to aphids than 'Zhongshuang11'. The results of the field survey showed that the number of aphids on 'Deleyou6' was significantly higher than that on 'Zhongshuang11' throughout the reproductive period of oilseed rape. The results of the study by Hao et al. [28] showed that 'Zhongshuang11' leaves had thicker upper epidermis and more trichomes compared with 'Deleyou6' leaves and there were impediments to aphid feeding at the leaf surface, mesophyll and phloem. These results indicate that 'Deleyou6' is an aphid-susceptible cultivar and 'Zhongshuang11' is an aphid-resistant cultivar. Therefore, these two cultivars were selected for this study to investigate aphid–-*S. sclerotiorum* interactions and the effects of the aphid resistance of the cultivar on these interactions. At the same time, following artificial inoculation with *S. sclerotiorum* to assess the resistance of the two cultivars, the results indicated that 'Deleyou6' and 'Zhongshuang11' exhibited medium resistance to *S. sclerotiorum*.

Following aphid feeding, *S. sclerotiorum* incidence was significantly increased in our laboratory trials. Moreover, we discovered that aphids were capable of transporting ascospores after being sprayed with ascospore suspensions and spreading them to healthy plants, leading to disease. Combined with the data from the 3-year field study, there was a significant positive correlation between aphid populations and *S. sclerotiorum* incidence. It can be concluded that aphids do promote the infection of *S. sclerotiorum*, although our study did not provide direct evidence. Microbiomes can be found in the insect's exoskeleton, gut, body, salivary glands and insect intercellular spaces but fungi can only attach to external body parts [60–63]. Shamshad and his team [64] recorded how *Lycoriella ingenua* (Dufour) (Diptera: Sciaridae) transmitted dry bubble disease, a disease that occurs frequently in mushroom farming and is caused by *Lecanicillium fungicola* (Preuss) Zare and Gams. Scanning electron microscopy (SEM) images consistently revealed fungal spores only on the femorotibial joint setae and tarsi of all flies introduced to *Trichoderma aggressivum* ft. *aggressivum* (Samuels and Gams) culture plates, with a small number of spores observed on the ovipositors. The flies visited the Petri plates inoculated with *T. aggressivum*, after being released into the chamber. Once landing, the exoskeleton of the flies could attach to the spores, either by passively or actively collecting. Subsequently, the flies visited the clean Petri dishes, resulting in green mould infections [65]. Ascospores of *S. sclerotiorum* are covered in sticky mucilage, which aids in the adhesion to the substrates. Senescent flower petals and wounded tissues are excellent sources of exogenous nutrients for ascospore germination [66]. It is hypothesised that aphids may also attach to fungal spores on their body surface and transfer these spores to healthy plants. Ascospore germination may be facilitated by nutrients provided by plant damage caused by aphid feeding or aphid honeydew.

According to the EPG results, we observed that *S. sclerotiorum* may directly (by ascospores) or indirectly affect the feeding behaviour of aphids. The duration required by aphids to commence their initial puncture on *S. sclerotiorum*-infected oilseed rape plants exhibited a notable reduction compared with uninfected oilseed rape. This observation implies that infestation by *S. sclerotiorum* decreased the plant's resistance or made it more appealing to aphids. Similar relations between fungi and insects have also demonstrated an increased attraction of plants to insects after fungal infection [67,68]. This attraction could be attributed to the volatile compounds released by the plant after pathogen infestation or by the pathogen. For example, *Fusarium verticillioides* (Saccardo) Nirenberg produces volatile compounds such as acetaldehyde, ethyl acetate and certain alcohols that have been proven to attract sap beetles (Coleoptera: Nitidulidae) [69]. However, clover root borers are attracted to diseased clover roots but not to isolates of Fusarium species causing root rot on clover [70]. Pathogens that can be transmitted by insects may have evolved strategies to attract insect vectors, resulting in increased insect attraction to infected hosts, potentially leading to amplified disease transmission and the prevalence of infective vectors [71,72].

Plant pathogens can also induce alterations in plant phloem, xylem, phytohormones and microelement ratios [73]. Our research has observed that aphids initiate intracellular penetration sooner and significantly shorten the duration of their pathway within the mesophyll infected with *S. sclerotiorum*, particularly in the case of the aphid-resistant cultivar 'Zhongshuang11'. In addition, aphids demonstrate a significantly higher frequency of brief probing and cell puncturing than uninfected controls. The observed changes align with those induced by turnip mosaic virus (TuMV) infection. However, as aphids serve as vectors for TuMV transmission and acquire the virus through cell puncture [28], they are not expected to acquire the fungus through cell puncture. *S. sclerotiorum* infection of plants is believed to modify the mesophyll structure, diminishing its resistance to aphids. The increased intracellular punctures also suggest the possibility that aphids extract small amounts of nutrients from the cell punctures, or facilitate the flow of cell contents to provide nutrients for further infestation by *S. sclerotiorum*.

After colonising the plant, *S. sclerotiorum* triggers the biosynthesis of both glucosinolates (GSLs) and indolic GSLs, which are linked to *S. sclerotiorum* resistance [74–76]. GSLs are significant secondary metabolites in cruciferous vegetables and are closely associated with biotic and abiotic stresses. Numerous studies have indicated that GSL content is positively correlated with resistance to diseases and insects [77–82]. When a plant cell is ruptured because of pest or pathogen attack, or mechanical wounding, GSLs and myrosinase (hydrolytic enzymes, M) come into contact and are hydrolysed in the presence of water to release various products, including isothiocyanates (ITCs) [83], which have a wide range of biocidal characteristics [84,85]. Despite this, *S. sclerotiorum* can still infect Brassica tissues. *S. sclerotiorum* develops tolerance to ITCs after initial exposure to sub-lethal doses [86]. However, these induced compounds may act as antixenosis agents against aphids. Our study found that in the phloem of infected plants, aphids reduced salivation before ingestion and decreased ingestion on the aphid-susceptible cultivar 'Deleyou6' and infection with *S. sclerotiorum* did not result in less aphid feeding on the cultivar 'Zhongshuang11'. 'Zhongshuang11', shown by Hao et al. [28], had phloem impeding factors, such as secondary metabolites, that defend against or reduce aphid feeding [87,88], compared with 'Deleyou6'. It also suggests that aphid feeding is maintained at a lower level after *S. sclerotiorum* infection of plants. This also suggests that the infected phloem is indeed unsuitable for aphid feeding or has an exclusionary effect on aphids, resulting in increased salivary secretion but reduced

feeding. Reduced aphid feeding on aphid-susceptible cultivars also prevents competition for nutrients with *S. sclerotiorum*. Because the phloem of 'Zhongshuang11' is relatively aphid resistant, aphids need to secrete a large amount of saliva to overcome the obstruction and feed relatively little. Therefore, after the infection of *S. sclerotiorum*, to satisfy the aphid's basic nutritional needs, aphid ingestion may not be further reduced. Similarly, ophiostomatoid (blue-stain fungus), which is carried on the surface of the body and in the gut of dispersing beetles, *Ips typographus* (L.) (Coleoptera: Curculionidae: Scolytinae), into breeding galleries in the phloem tissue of Norway spruce, *Picea abies* (L.) Karst. (Pinales: Pinaceae), can assist beetles in overcoming and exhausting host defences but can induce terpene and phenolic accumulation to strongly inhibit subsequent spruce bark beetle colonisation [89]. Lahr and Krokene [90] suggested that the effects of fungal resource consumption are unlikely to provide direct nutritional benefits to developing beetle larvae when *I. typographus* vectors *Ceratocystis polonica* (Siemaszko) C. Moreau. Because they did not observe an increase in phloem resource concentrations, such as nitrogen, following fungal inoculation.

S. sclerotiorum ascospores, after adhering to the aphid body surface, also affected aphid feeding behaviour, but this effect differed in different cultivars. In the aphid-susceptible cultivar 'Deleyou6', aphids carrying ascospores had a significantly longer time to start their first penetration compared with aphids not carrying ascospores, and in the aphid-resistant cultivar 'Zhongshuang11', the time for aphids carrying ascospores to start the first penetration was significantly shorter. Because the aphids had not yet started probing the plants, there were no changes in the plants and only the aphids' body surfaces carrying the ascospores made a difference. Therefore, it can be speculated that the aphids' feeding behaviour was affected by the ascospores adhering to the aphids' body surfaces. The EPG study was replicated for at least 30 aphids in each treatment, and if ascospores hindered the aphids from feeding because of their adherence to the aphids' stylets, then the aphids carrying ascospores should have delayed the time of the first penetration, regardless of whether they were in the aphid-resistant or aphid-susceptible cultivars. However, the results showed that this time was significantly reduced in the resistant cultivars, implying that the ascospores, wherever they adhered to the aphids, were not simply physically hindered, but perhaps, there are more complex actively regulated processes of perceived cultivar differences that need to be further investigated.

After being attacked by herbivores, plants undergo a modification of their cell walls [91], which includes receiving signals released by insects through receptors that activate the plant's immune system [92,93]. In addition, various alterations occur in the plasma membrane of plant cells. Chemical cues found in herbivore oral secretions or oviposition fluid induce herbivore-associated molecular patterns (HAMPs) [94]. These HAMP compounds also trigger the release of leaf volatiles [95]. Herbivore attacks cause changes in plasma membrane potential (Vm), followed by the generation of secondary messengers such as [Ca^{2+}] cyt and reactive oxygen species (ROS) [94], as well as a rapid increase in phytohormones [96]. In the mesophyll, aphids carrying ascospores puncture cells earlier, which increases the frequency and duration of short probes and cell punctures, along with shorter pathway durations. The increased obstructions indicate faster and more frequent penetration of mesophyll cells by aphids [97]. These changes resemble the effects of TuMV infection on aphid feeding behaviour [28], but it is unlikely that aphids carrying ascospores inoculate the fungus through cell puncture. Instead, the aphids may puncture the cells to release the necessary nutrients and create a suitable environment for the germination of ascospores. In this study, we allowed aphids to carry ascospores by spraying ascospore suspensions, and perhaps, the ascospores also entered the aphid's mouthparts. Whether the ascospores stayed on the leaf surface after the aphid began to puncture the leaf or entered the leaf with the aphid's stylets was unclear in our study. The differences were mainly in whether the aphids carried ascospores or not and the resulting changes in feeding behaviour, such as increased frequency of brief probing and intracellular puncturing by aphids carrying ascospores, which may be affected by the complex triple interactions of the *S. sclerotiorum* ascospores with aphids and plants. However, we hypothesise that such behavioural changes are conducive to *S. sclerotiorum* colonisation and development.

Plants accumulate secondary metabolites in response to herbivore attacks, such as phenolic compounds and tannins [98–101]. Phenolic compounds, including lignin, coumarins, furanocoumarins, flavonoids, and tannins, serve as deterrents and toxins for feeding, thereby reducing the nutritional value of plant food [102,103]. In this study, aphids carrying ascospores showed increased frequency and duration of total salivation (n_E1 and s_E1), reduced salivation before the first sustained feeding (d_E1followedby1sE2) and decreased ingestion in the phloem. Lahr and Krokene [90] found that bark beetles and fungi have a mutualistic relation. However, beetles may compete with fungi for food resources within the tree if they consume phloem tissue before it is colonised by fungi. Therefore, aphids carrying ascospores may decrease feeding, which could help in avoiding competition for plant nutrients with *S. sclerotiorum* infestations.

The impact of *S. sclerotiorum* on aphid feeding behaviour was also influenced by the oilseed rape cultivar. Variations in aphid resistance between 'Deleyou6' and 'Zhongshuang11' led to differences in *S. sclerotiorum*-induced changes in aphid feeding behaviour. In cultivars with different aphid resistance, the effect of *S. sclerotiorum* on aphid feeding behaviour tended to weaken the plant's own resistance. This means that the resistance of aphid-resistant cultivars was weakened by *S. sclerotiorum* infection and that of aphid-susceptible cultivars was strengthened. Despite changes in resistance, the effect of *S. sclerotiorum* on aphid feeding behaviour appears to promote the spread of *S. sclerotiorum*. Aphids penetrate cells to provide nutrients for *S. sclerotiorum* infection and ascospore germination. They also secrete saliva to reduce plant resistance to *S. sclerotiorum* development. Reduced ingestion by aphids, in turn, avoids competition for nutrients with *S. sclerotiorum*. It seems that the effects of *S. sclerotiorum* on aphid feeding behaviour favour the propagation and development of *S. sclerotiorum* and the benefits to the aphids are minor or that *S. sclerotiorum* infestation may also reduce plant resistance to aphid feeding and provide better nutrients for aphid feeding, which requires further research for confirmation. There are several examples where fungi appear to deceive insects (for review: see Ref. [104]). These fungi use olfactory and gustatory cues to attract insects to spore-rich areas, but the attracted insects do not receive any nectar reward or future fitness benefits through this spore dispersal process. Even in some insect vectors for various phytopathogenic fungi, researchers have observed reduced survival, fecundity, biomass, and slower development. Therefore, the interaction between *S. sclerotiorum* and aphids may not be a

mutually beneficial relation.

5. Conclusions

There is indeed a correlation between aphids and *S. sclerotiorum*. There is indirect evidence that aphids are capable of transmitting and depositing *S. sclerotiorum* ascospores onto healthy plants, leading to the development of *S. sclerotiorum*. *S. sclerotiorum* incidence has been observed to increase after aphid feeding. Insects serve as vectors for plant pathogens, as evidenced by close associations between insects and infected plants, regular insect visits to wounded healthy plants, the presence of the pathogen on the insect following visitation to a diseased plant, and the subsequent development of diseases on plants after visits by pathogen-infested insects [105]. To investigate the potential of aphids to carry *S. sclerotiorum* ascospores, we will further explore the attachment of ascospores to the outer integument of the aphid and will determine if this attachment is specific to a certain part of the body. In addition, we will analyse the abundance of ascospores on the body surface of aphids. Furthermore, we will investigate whether there are specific deposition sites on healthy plants for ascospores carried by aphids and whether infestation occurs directly into the plant or via aphid feeding sites.

S. sclerotiorum can affect aphid probing behaviour in multiple ways: directly by being carried by aphids or indirectly by infecting the host plant. This influence is specific and varies in a manner that promotes the spread of *S. sclerotiorum*. This symbiotic relation is similar to that between TuMV and its vector aphids [28]. These findings indicate that aphids might aid in the dissemination of *S. sclerotiorum*, but it remains uncertain whether *S. sclerotiorum* offers any advantages to aphids. Additional physiological, biochemical and molecular experiments are necessary to validate this. Although cultivar resistance to aphids affects the regulatory impact of *S. sclerotiorum* on aphid feeding behaviour, it does not alter the fact that this regulation uniformly favours *S. sclerotiorum* transmission and infestation.

Data availability statement

The associated data produced for this study can be cited: Zhong-Ping Hao. (2024), "Facilitation of *Sclerotinia sclerotiorum* infestation by aphid feeding behaviour is not affected by aphid resistance in oilseed rape", Mendeley Data, V2, https://doi.org/10.17632/bz8n3txssy.2.

Funding

This research was funded by the Earmarked Fund for China Agriculture Research System, grant number CARS-12 and the Achievement Transformation Project of Anhui Academy of Agricultural Sciences: Establishment and Demonstration of Oilseed Rape Industry Chain Development Mode, grant number 2024YL023.

CRediT authorship contribution statement

Zhong-Ping Hao: Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Zeng-Bei Feng:** Visualization, Validation, Software, Investigation, Formal analysis. **Lei Sheng:** Writing – original draft, Methodology, Data curation. **Wei-Xin Fei:** Validation, Resources. **Shu-Min Hou:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Prof. Li Qiangsheng (the Crop Research Institute, Anhui Academy of Agricultural Sciences, China) for providing a strain of *S. sclerotiorum* and advice on *S. sclerotiorum* studies.

References

[1] M.C. Derbyshire, M. Denton-Giles, The control of Sclerotinia stem rot on oilseed rape (*Brassica napus*): current practices and future opportunities, Plant Pathol. 65 (2016) 859–877.
[2] M.A.K.W.C. Surinder, S. Banga, M.P.Y.V.T. Baudh, B.M.J. Barbetti, Patterns of inheritance for cotyledon resistance against *Sclerotinia sclerotiorum* in *Brassica napus*, Euphytica 216 (2020) 79.
[3] Q. Yang, W. Liu, C. Huang, Statistics and analysis of oilseed rape losses caused by main diseases and insect pests in recent 10 years, Plant Prot. 44 (3) (2018) 24–30.
[4] P. Sharma, P. Meena, P. Verma, G. Saharan, N. Mehta, D. Singh, A. Kumar, *Sclerotinia sclerotiorum* (lib) de Bary causing sclerotinia rot in oilseed Brassicas: a review, J. Oilseed Brassica 1 (2016) 1–44.
[5] P. Duarte, L. Menze, G.N. Abdelrasoul, S. Yosinski, Z. Kobos, R. Stuermer, M. Reed, J. Yang, S. Li, J. Chen, Single ascospore detection for the forecasting of Sclerotinia stem rot of canola, Lab Chip 20 (2020) 3644–3652, https://doi.org/10.1039/D0LC00426J.

[6] M.D. Bolton, B.P.H.J. Thomma, B.D. Nelson, *Sclerotinia sclerotiorum* (Lib.) de Bary: Biology and molecular traits of a cosmopolitan pathogen, Mol. Plant Pathol. 7 (2006) 1–16.

[7] Y. Yuan, S. Guo, G. Yang, B. Dan, Advances in research on rape Sclerotia and resistance breeding, Asian Agr. Res. 12 (7) (2020) 55–60, https://doi.org/10.19601/j.cnki.issn1943-9903.2020.07.014.

[8] I. Jamaux, B. Gelie, C. Lamarque, Early stages of infection of rapeseed petals and leaves by *Sclerotinia sclerotiorum* revealed by scanning electron microscopy, Plant Pathol. 44 (1995) 22–30.

[9] Z.W. Luo, J. Lv, Studies on the control of *Sclerotinia sclerotiorum* at seedling stage under different treatment conditions of oilseed rape seeds before sowing, Heilongjiang Sci. Technol. Inf. 22 (2011) 261.

[10] K.O. Fening, E.E. Forchibe, F.O. Wamonje, I. Adama, K. Afreh-Nuamah, J.P. Carr, First report and distribution of the indian mustard aphid, *Lipaphis erysimi pseudobrassicae* (Hemiptera: Aphididae) on cabbage (*Brassica oleracea* var *capitata*) in Ghana, J. Econ. Entomol. 113 (2020) 1363–1372.

[11] T. Gao, Z. Wang, H. Hu, Prelimiary study on insects carrying *Sclerotinia Sclerotiorum*, J. Anhui Agric. Univ. 23 (4) (1996) 493–495.

[12] E. Yamoah, A Model System Using Insects to Vector *Fusarium Tumidum* for Biological Control of Gorse (*Ulex Europaeus*), Lincoln University, Canterbury, New Zealand, 2007. Doctor's Thesis.

[13] Y. Lu, L. Sui, Z. Yang, G. Mao, W.-J. Xu, Y. Zhao, L. Li, Q.-Y. Li, Z.-K. Zhang, Synergistic control against Asian corn borer (ACB) by *Trichogramma* vectored *Beauveria bassiana* infection on survival larvae escaped from parasitism. IOP Conf. Series: earth Env, Sci. 547 (2020) 012021, https://doi.org/10.1088/1755-1315/547/1/012021.

[14] L. De Marzo, S. Frisullo, F. Lops, V. Rossi, Possible dissemination of *Spilocaea olegina* conidia by insects (*Ectopsocus briggsi*), EPPO Bull. 23 (1993) 389–391.

[15] S.A. Syazwan, A. Mohd-Farid, W.-A. Wan-Muhd-Azrul, H.M. Syahmi, A.M. Zaki, S.P. Ong, R. Mohamed, Survey, identification, and pathogenicity of *Ceratocystis fimbriata* complex associated with wilt disease on *Acacia mangium* in Malaysia, Forests 12 (2021) 1782, https://doi.org/10.3390/f12121782.

[16] M.P. de Nooij, The role of weevils in the infection process of the fungus *Phomopsis subordinaria* in *Plantago lanceolata*, Oikos 52 (1) (1988) 51–58.

[17] M. Fermaud, R. LeMenn, Association of *Botrytis cinerea* with grape berry moth larvae, Phytopathology 79 (1989) 651–656.

[18] M. Fermaud, R.I. Menn, Menn R. Le, Transmission of *Botrytis cinerea* to grapes by grape berry moth larvae, Phytopathology 82 (1992) 1393–1398.

[19] R. Isaacs, K.S. Mason, E. Maxwell, Stage-specific control of grape berry moth, *Endopiza viteana* (Clemens) (Lepidoptera: Tortricidae), by selective and broad spectrum insecticides, J. Econ. Entomol. 98 (2005) 415–422, https://doi.org/10.1093/jee/98.2.415.

[20] T.A. Klein, B.A. Auld, Wounding can improve efficacy of *Colletotrichum orbiculare* as a mycoherbicide for Bathurst burr, Aust. J. Exp. Agric. 36 (1996) 185–187, https://doi.org/10.1071/EA9960185.

[21] P.E. Hatcher, Three-way interactions between plant pathogenic fungi, herbivorous insects and their host plants, Biol. Rev. 70 (1995) 639–694.

[22] D.C. Murray, D.R. Walters, Increased photosynthesis and resistance to rust infection in upper, uninfected leaves of rusted broad bean (*Vicia faba*), New Phytol. 120 (1992) 235–242.

[23] P. Claudel, Q. Chesnais, Q. Fouché, C. Krieger, D. Halter, F. Bogaert, S. Meyer, S. Boissinot, P. Hugueney, V. Ziegler-Graff, A. Ameline, V. Brault, The aphid-transmitted turnip yellows virus differentially affects volatiles emission and subsequent vector behavior in two *Brassicaceae* plants, Int. J. Mol. Sci. 19 (8) (2018) 2316, https://doi.org/10.3390/ijms19082316.

[24] A. Bak, J.B. Emerson, Cauliflower mosaic virus (CaMV) biology, management, and relevance to GM plant detection for sustainable organic agriculture, Front. Sustain. Food Syst. 4 (2020) 21, https://doi.org/10.3389/fsufs.2020.00021.

[25] D.P. Zuo, M.J. He, X.R. Chen, R.J. Hu, T.Y. Zhao, X.Y. Zhang, Y.M. Peng, Y. Wang, D.W. Li, J.L. Yu, C.G. Han, A simple method for the acquisition and transmission of Brassica yellows virus from transgenic plants and frozen infected leaves by aphids, Plants 10 (9) (2021) 1944, https://doi.org/10.3390/plants10091944.

[26] Q. Chesnais, V. Golyaev, A. Velt, C. Rustenholz, V. Brault, M.M. Pooggin, M. Drucker, Comparative plant transcriptome profiling of *Arabidopsis thaliana* Col-0 and *Camelina sativa* var. Celine infested with *Myzus persicae* aphids acquiring circulative and noncirculative viruses reveals virus- and plant-specific alterations relevant to aphid feeding behavior and transmission, Microbiol. Spectr. 10 (4) (2022) e0013622, https://doi.org/10.1128/spectrum.00136-22.

[27] S.F. Greer, A. Surendran, M. Grant, R. Lillywhite, The current status, challenges, and future perspectives for managing diseases of brassicas, Front. Microbiol. 14 (2023) 1209258, https://doi.org/10.3389/fmicb.2023.1209258.

[28] Z.-P. Hao, Z.-B. Feng, L. Sheng, W.-X. Fei, S.-M. Hou, Aphids on aphid-susceptible cultivars have easy access to turnip mosaic virus, and effective inoculation on aphid-resistant cultivars of oilseed rape (*Brassica napus*), Plants 12 (2023) 1972, https://doi.org/10.3390/plants12101972.

[29] W.F. Tjallingii, Salivary secretions by aphids interacting with proteins of phloem wound responses, J. Exp. Bot. 57 (2006) 739–745.

[30] W.F. Tjallingii, Th Hogen Esch, Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals, Physiol. Entomol. 18 (1993) 317–328, https://doi.org/10.1111/j.1365-3032.1993.tb00604.x.

[31] T. Will, W.F. Tjallingii, A. Thonnessen, A.J.E. van Bel, Molecular sabotage of plant defence by aphid saliva, Proc. Natl. Acad. Sci. USA 104 (2007) 10536–10541.

[32] M.M. Kamal, S. Savocchia, K.D. Lindbeck, G.J. Ash, Biology and biocontrol of *Sclerotinia sclerotiorum* (Lib.) de Bary in oilseed Brassicas, Australas. Plant Pathol. 45 (2016) 1–14, https://doi.org/10.1007/s13313-015-0391-2.

[33] H.Q. Liu, M. Xie, Field management of oilseed rape cultivation and control measures of *Sclerotinia Sclerotiorum*, Farmers Consult 22 (2021) 21–22.

[34] E. Boszoradova, J. Libantova, I. Matusikova, Z. Poloniova, M. Jopcik, M. Berenyi, J. Moravcikova, Agrobacterium-mediated genetic transformation of economically important oilseed rape cultivars. Plant Cell Tiss, Organ Cult 107 (2011) 317–323, https://doi.org/10.1007/s11240-011-9982-y.

[35] S. Seifbarghi, M.H. Borhan, Y. Wei, C. Coutu, S.J. Robinson, D.D. Hegedus, Changes in the *Sclerotinia sclerotiorum* transcriptome during infection of *Brassica napus*, BMC Genom. 18 (2017) 266, https://doi.org/10.1186/s12864-017-3642-5.

[36] Z.P. Hao, S.M. Hou, B.C. Hu, F. Huang, X.L. Dang, Assessment of probing behavior of the cabbage aphid, *Brevicoryne brassicae* (Hemiptera: aphididae), on three *Brassica napus* cultivars at three developmental stages using Electropenetrography (EPG), J. Kans. Entomol. Soc. 90 (2017) 11–23.

[37] Z.P. Hao, H.X. Zhan, Y.L. Wang, S.M. Hou, How cabbage aphids *Brevicoryne brassicae* (L.) make a choice to feed on *Brassica napus* cultivars, Insects 10 (2019) 75, https://doi.org/10.3390/insects10030075.

[38] J. George, N.E. Jenkins, S. Blanford, M.B. Thomas, T.C. Baker, Malaria mosquitoes attracted by fatal fungus, PLoS One 8 (5) (2013) e62632, https://doi.org/10.1371/journal.pone.0062632.

[39] I.S. Qandah, Epidemiological Studies on Sclerotinia Stem Rot Caused by *Sclerotinia sclerotiorum* (Lib.) de Bary on Canola, North Dakota State University, Fargo, North Dakota, 2008. Doctor's Thesis.

[40] B.R. Ziesman, T.K. Turkington, U. Basu, S.E. Strelkov, A quantitative PCR system for measuring *Sclerotinia sclerotiorum* in canola (*Brassica napus*), Plant Dis. 100 (2016) 984–990, https://doi.org/10.1094/PDIS-05-15-0605-RE.

[41] J. Mei, L. Qian, J.O. Disi, X. Yang, Q. Li, J. Li, M. Frauen, D. Cai, W. Qian, Identification of resistant sources against *Sclerotinia sclerotiorum* in Brassica species with emphasis on *B. oleracea*, Euphytica 177 (2011) 393–399, https://doi.org/10.1007/s10681-010-0274-0.

[42] J. Mei, D. Wei, J.O. Disi, Y. Ding, Y. Liu, W. Qian, Screening resistance against *Sclerotinia sclerotiorum* in Brassica crops with use of detached stem assay under controlled environment, Eur. J. Plant Pathol. 134 (2012) 599–604, https://doi.org/10.1007/s10658-012-0040-3.

[43] Z.H. Zhu, Identification and evaluation of *Sclerotinia sclerotiorum* resistant varieties for oilseed rape, Anhui Agri. Sci. Bull. 24 (15) (2018) 50–52, 57.

[44] M.B. Uloth, M.P. You, M.J. Barbetti, Host resistance to Sclerotinia stem rot in historic and current *Brassica napus* and *B. juncea* varieties: critical management implications, Crop Pasture Sci. 66 (2015) 841–848, https://doi.org/10.1071/CP15064.

[45] J. Zhao, L. Buchwaldt, S.R. Rimmer, A. Sharpe, L. Mcgregor, D. Bekkaoui, D. Hegedus, Patterns of differential gene expression in *Brassica napus* cultivars infected with *Sclerotinia sclerotiorum*, Mol. Plant Pathol. 10 (5) (2009) 635–649.

[46] S.R. Rimmer, H.R. Kutcher, R.A.A. Morrall, Diseases of canola and mustard, in: K.L. Bailey, B.D. Gossen, R.K. Gugel, R.A.A. Morrall (Eds.), Diseases of Field Crops in Canada, University Extension Press, 2003, pp. 129–146.

[47] Y.L. Qin, Y.H. Cao, P.B. Zheng, W. Zhou, Comparison of *Sclerotinia sclerotiorum* resistance in different oilseed rape varieties, Hubei Plant Prot 178 (1) (2020) 31–32, 35.

[48] R.S.D. Al-Shindah, A.A. Hassan, M.S. Mansour, Isolation and identification of entomopathogenic fungi from of green peach sphid *Myzus Persicae* and evaluation of their activity for insect control, IOP Conf. Ser. Earth Environ. Sci. 1060 (2022) 012093, https://doi.org/10.1088/1755-1315/1060/1/012093.
[49] W.F. Tjallingii, Electrical recording of stylet penetration activities, in: A.K. Minks, P. Harrewijn (Eds.), Aphids, Their Biology, Natural Enemies and Control, Elsevier, 1988, pp. 95–108.
[50] K. Xue, X.-Y. Wang, C.-H. Huang, R.-J. Wang, B. Liu, F.-M. Yan, C.-R. Xu, Stylet penetration behaviors of the cotton aphid *Aphis gossypii* on transgenic Bt cotton, Insect Sci. 16 (2009) 137–146.
[51] B. Gabrys, M. Pawluk, Acceptability of different species of Brassicaceae as hosts for the cabbage aphid, Entomol. Exp. Appl. 91 (1999) 105–109.
[52] E. Sarria, M. Cid, E. Garzo, A. Fereres, Excel workbook for automatic parameter calculation of EPG data, Comput. Eletron. Agric. 67 (2009) 35–42.
[53] N. Zamani-Noor, Baseline sensitivity and control efficacy of various group of fungicides against *Sclerotinia sclerotiorum* in oilseed rape cultivation, Agronomy 11 (2021) 1758, https://doi.org/10.3390/agronomy11091758.
[54] I.H. Chapela, S.A. Rehner, T.R. Schultz, U.G. Mueller, Evolutionary history of the symbiosis between fungus growing ants and their fungi, Science 266 (1994) 1691–1694.
[55] B.D. Farrell, A.S. Sequeira, B.C. O'Meara, B.B. Normark, J.H. Chung, B.H. Jordal, The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae), Evolution 55 (2001) 2011–2027.
[56] D.K. Aanen, P. Eggleton, C. Rouland-Lefevre, T. GuldbergFroslev, S. Rosendahl, J.J. Boomsma, The evolution of fungus-growing termites and their mutualistic fungal symbionts, Proc. Natl. Acad. Sci. USA 99 (2002) 14887–14892.
[57] M.F. Madelin, Fungal parasites of insects, Annu. Rev. Entomol. 11 (1966) 423–448.
[58] R. Hofstetter, J. Cronin, K. Klepzig, J. Moser, M. Ayres, Antagonisms, mutualisms and commensalisms affect outbreak dynamics of the southern pine beetle, Oecologia 147 (2006) 679–691.
[59] B. Wang, C. Salcedo, M. Lu, J. Sun, Mutual interactions between an invasive bark beetle and its associated fungi, Bull. Entomol. Res. 102 (2012) 71–77.
[60] V. Brault, M. Uzest, B. Monsion, E. Jacquot, S. Blanc, Aphids as transport devices for plant viruses, C. R. Biol. 333 (6–7) (2010) 524–538, https://doi.org/10.1016/j.crvi.2010.04.001.
[61] P. Engel, N.A. Moran, The gut microbiota of insects- diversity in structure and function, FEMS Microbiol. Rev. 37 (2013) 699–735, https://doi.org/10.1111/1574-6976.12025.
[62] A.K. Hansen, N.A. Moran, The impact of microbial symbionts on host plant utilization by herbivorous insects, Mol. Ecol. 23 (2013) 14731496, https://doi.org/10.1111/mec.12421.
[63] C.W. McCoy, Pathogens of eriophyoid mites, in: E.E. Lindquist, M.W. Sabelis, J. Bruin (Eds.), Eriophyoid Mites—Their Biology, Natural Enemies and Control, Elsevier, 1996, pp. 481–490.
[64] A. Shamshad, A.D. Clift, S. Mansfield, The effect of tibia morphology on vector competency of mushroom sciarid flies, J. Appl. Entomol. 133 (2009) 484–490.
[65] M. Mazin, R. Harvey, S. Andreadis, J. Pecchia, K. Cloonan, E.G. Rajotte, Mushroom sciarid fly, *Lycoriella ingenua* (Diptera: Sciaridae) adults and larvae vector mushroom green mold (*Trichoderma aggressivum ft. aggressivum*) spores, Appl. Entomol. Zool. 54 (2019) 369–376, https://doi.org/10.1007/s13355-019-00632-2.
[66] M.D. Bolton, B.P.H.J. Thomma, B.D. Nelson, *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen, Mol. Plant Pathol. 7 (2006) 1–16, https://doi.org/10.1111/j.1364-3703.2005.00136.x.
[67] S.E. Braun, J.P. Sanderson, M.L. Daughtrey, S.P. Wraight, Attraction and oviposition responses of the fungus gnat Bradysia impatiens to microbes and microbe-inoculated seedlings in laboratory bioassays, Entomol. Exp. Appl. 145 (2012) 89–101.
[68] G. McLeod, R. Gries, S.H. von Reuss, J.E. Rahe, R. McIntosh, W.A. König, G. Gries, The pathogen causing Dutch elm disease makes host trees attract insect vectors, P. Roy. Soc. B-Biol. Sci. 272 (2005) 2499–2503, https://doi.org/10.1098/rspb.2005.3202.
[69] R.J. Bartelt, D.T. Wicklow, Volatiles from *Fusarium verticillioides* (sacc.) nirenb. And their attractiveness to nitidulid beetles, J. Agric. Food Chem. 47 (1999) 2447–2454.
[70] K.T. Leath, R.A. Byers, Attractiveness of diseased red clover roots to the clover root borer, Phytopathology 63 (1973) 428–431.
[71] X. Martini, D.S. Willett, E.H. Kuhns, L.L. Stelinski, Disruption of vector host preference with plant volatiles may reduce spread of insect-transmitted plant pathogens, J. Chem. Ecol. 42 (2016) 357–367.
[72] R.M. Smith, V. Alonso-Chavez, J. Helps, M.W. Shaw, F. van den Bosch, Modelling lifestyle changes in insect endosymbionts, from insect mutualist to plant pathogen, Evol. Ecol. 34 (2020) 867–891, https://doi.org/10.1007/s10682-020-10071-z.
[73] S. Berger, A.K. Sinha, T. Roitsch, Plant physiology meets phytopathology: plant primary metabolism and plant-pathogen interactions, J. Exp. Bot. 58 (2007) 4019–4026.
[74] J. Wu, Q. Zhao, Q. Yang, H. Liu, Q. Li, X. Yi, Y. Cheng, L. Guo, C. Fan, Y. Zhou, Comparative transcriptomic analysis uncovers the complex genetic network for resistance to *Sclerotinia sclerotiorum* in *Brassica napus*, Sci. Rep.-UK 6 (2016) 19007, https://doi.org/10.1038/srep19007.
[75] J. Zhao, A.J. Peltier, J. Meng, T.C. Osborn, C.R. Grau, Evaluation of Sclerotinia stem rot resistance in oilseed *Brassica napus* using a petiole inoculation technique under greenhouse conditions, Plant Dis. 88 (2004) 1033–1039.
[76] A. Borges, A.C. Abreu, C. Ferreira, M.J. Saavedra, L.C. Simoes, M. Simoes, Antibacterial activity and mode of action of selected glucosinolate hydrolysis products against bacterial pathogens, J. Food Sci. Technol. 52 (2015) 4737–4748.
[77] J. Kroymann, S. Donnerhacke, D. Schnabelrauch, T. Mitchell-Olds, Evolutionary dynamics of an Arabidopsis insect resistance quantitative trait locus, Proc. Natl. Acad. Sci. USA 100 (2003) 14587–14592.
[78] P.-H. Dubuis, C. Marazzi, E. Städler, F. Mauch, Sulphur deficiency causes a reduction in antimicrobial potential and leads to increased disease susceptibility of oilseed rape, J. Phytopathol. 153 (2005) 27–36.
[79] F. Kuhlmann, C. Muller, Independent responses to ultraviolet radiation and herbivore attack in Broccoli, J. Exp. Bot. 60 (2009) 3467–3475.
[80] P. Barah, P. Winge, A. Kusnierczyk, D.H. Tran, A.M. Bones, Molecular signatures in *Arabidopsis Thaliana* in response to insect attack and bacterial infection, PLoS One 8 (2013) e58987.
[81] A.H.K. Robin, R. Laila, M. Abuyusuf, J.I. Park, I.S. Nou, *Leptosphaeria maculans* alters glucosinolate accumulation and expression of aliphatic and indolic glucosinolate biosynthesis genes in blackleg disease-resistant and -susceptible cabbage lines at the seedling stage, Front. Plant Sci. 11 (2020) 1134.
[82] Q. Sun, E. Zhang, Y. Liu, Z. Xu, M. Hui, X. Zhang, M. Cai, Transcriptome analysis of two lines of *Brassica Oleracea* in response to early infection with *Xanthomonas Campestris* pv, Campestris. Can. J. Plant Pathol 43 (2020) 127–139.
[83] A.P. Vig, G. Rampal, T.S. Thind, S. Arora, Bio-protective effects of glucosinolates–A review, LWT–Food Sci. Technol. 42 (2009) 1561–1572, https://doi.org/10.1016/j.lwt.2009.05.023.
[84] Ş. Kurt, U. Güneş, E.M. Soylu, In vitro and in vivo antifungal activity of synthetic pure isothiocyanates against *Sclerotinia sclerotiorum*, Pest Manag. Sci. 67 (2011) 869–875, https://doi.org/10.1002/ps.2126.
[85] R. Warmington, J.P. Clarkson, Volatiles from biofumigant plants have a direct effect on carpogenic germination of sclerotia and mycelial growth of *Sclerotinia sclerotiorum*, Plant Soil 401 (2016) 213–229, https://doi.org/10.1007/s11104-015-2742-8.
[86] S. Rahmanpour, D. Backhouse, H.M. Nonhebel, Induced tolerance of *Sclerotinia sclerotiorum* to isothiocyanates and toxic volatiles from Brassica species, Plant Pathol. 58 (2009) 479–486.
[87] S.A. Zebelo, M.E. Maffei, Role of early signalling events in plant-insect interactions, J. Exp. Bot. 66 (2015) 435–448.
[88] T. Belete, Defense mechanisms of plants to insect pests: from morphological to biochemical approach, Trends Tech. Sci. Res. 2 (2018) 555584.
[89] T. Zhao, P. Krokene, J. Hu, E. Christiansen, N. Björklund, B. Långström, H. Solheim, A.-K. Borg-Karlson, Induced terpene accumulation in Norway spruce inhibits bark beetle colonization in a dose-dependent manner, PLoS One 6 (10) (2011) e26649, https://doi.org/10.1371/journal.pone.0026649.
[90] E.C. Lahr, P. Krokene, Conifer stored resources and resistance to a fungus associated with the spruce bark beetle *Ips typographus*, PLoS One 8 (8) (2013) e72405, https://doi.org/10.1371/journal.pone.0072405.

[91] K.J. Kloth, I.N. Abreu, N. Delhomme, I. Petrík, C. Villard, C. Ström, F. Amini, O. Novák, T. Moritz, B.R. Albrectsen, PECTIN AceTyLeSTerase9 affects the transcriptome and metabolome and delays aphid feeding, Plant Physiol. 181 (2019) 1704–1720.
[92] T. Sun, Y. Zhang, Short- and long-distance signaling in plant defense, Plant J. 105 (2021) 505–517.
[93] S. Mostafa, Y. Wang, W. Zeng, B. Jin, Plant responses to herbivory, wounding, and infection, Int. J. Mol. Sci. 23 (2022) 7031, https://doi.org/10.3390/ijms23137031.
[94] A. Gandhi, R.R. Kariyat, C. Chappa, M. Tayal, N. Sahoo, Tobacco hornworm (*Manduca sexta*) oral secretion elicits reactive oxygen species in isolated tomato protoplasts, Int. J. Mol. Sci. 21 (2020) 8297.
[95] P. Reymond, Receptor kinases in plant responses to herbivory, Curr. Opin. Biotechnol. 70 (2021) 143–150.
[96] H. Si, H. Liu, Y. Sun, Z. Xu, S. Liang, B. Li, X. Ding, J. Li, Q. Wang, L. Sun, X. Zhang, S. Jin, Transcriptome and metabolome analysis reveal that oral secretions from *Helicoverpa armigera* and *Spodoptera litura* influence wound-induced host response in cotton, Crop J. 8 (2020) 929–942, https://doi.org/10.1016/j.cj.2019.12.007.
[97] Z.-P. Hao, H.-X. Zhan, L.-L. Gao, F. Huang, L.-N. Zhu, S.-M. Hou, Possible effects of leaf tissue characteristics of oilseed rape *Brassica napus* on probing and feeding behaviors of cabbage aphids *Brevicoryne brassicae*, Arthropod-Plant Inte. 14 (2020) 733–744, https://doi.org/10.1007/s11829-020-09782-5.
[98] I.T. Baldwin, J.C. Schultz, Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants, Science 221 (1983) 277–279.
[99] H.J. Zeringue, Changes in cotton leaf chemistry induced by volatile elicitors, Phytochemistry 26 (1987) 1357–1360.
[100] D. Choi, R.M. Bostock, S. Avdiushko, D.F. Hildebrand, Lipid-derived signals that discriminate wound- and pathogen-responsive isoprenoid pathways in plants: methyl jasmonate and the fungal elicitor arachidonic acid induce different 3-hydroxy-3-methylglutaryl-coenzyme a reductase genes and antimicrobial isopre, Proc. Natl. Acad. Sci. USA 91 (1994) 2329–2333.
[101] S.A. Zebelo, M.E. Maffei, Role of early signalling events in plant-insect interactions, J. Exp. Bot. 66 (2015) 435–448.
[102] T. Belete, Defense mechanisms of plants to insect pests: from morphological to biochemical approach, Trends Tech. Sci. Res. 2 (2018) 555584.
[103] M. Gantner, A. Najda, D. Piesik, Effect of phenolic acid content on acceptance of hazel cultivars by filbert aphid, Plant Protect. Sci. 55 (2019) 116–122.
[104] K.R. Cloonan, Behaviorally Active Substances Affecting Reproductive Success of the Fungus Gnat, *Lycoriella Ingenua*, a Pest of White Button Mushrooms, *Agaricus Bisporus*, Pennsylvania State University, Commonwealth of Pennsylvania, USA, 2017 (Doctor's Thesis.
[105] D.H. Lee, J. Roux, B.D. Wingfield, M.J. Wingfield, A microsatellite-based identification tool used to confirm vector association in a fungal tree pathogen, Australas. Plant Pathol. 47 (2018) 63–69, https://doi.org/10.1007/s13313-017-0535-7.