Received: 22 March 2019 | Revised: 24 May 2019 | Accepted: 6 July 2019

DOI: 10.1111/jen.12677

ORIGINAL CONTRIBUTION

JOURNAL OF APPLIED ENTOMOLOGY WILEY

Resistance to three thrips species in *Capsicum* spp. depends on site conditions and geographic regions

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Funding information
Toegepaste en Technische Wetenschappen - NWO, Grant/Award Number: 13552

Abstract

Capsicum species are commercially grown for pepper production. This crop suffers severely from thrips damage and the identification of natural sources of thrips resistance is essential for the development of resistant cultivars. It is unclear whether resistance to *Frankliniella occidentalis* as assessed in a specific environment holds under different conditions. Additionally, other thrips species may respond differently to the plant genotypes. Screening for robust and general resistance to thrips encompasses testing different *Capsicum* accessions under various conditions and with different thrips species. We screened 11 *Capsicum* accessions (*C. annuum* and *C. chinense*) for resistance to *F. occidentalis* at three different locations in the Netherlands. Next, the same 11 accessions were screened for resistance to *Thrips palmi* and *Scirtothrips dorsalis* at two locations in Asia. This resulted in a unique analysis of thrips resistance in *Capsicum* at five different locations around the world. Finally, all accessions were also screened for resistance to *F. occidentalis* in the Netherlands using a leaf disc choice assay, allowing direct comparison of whole plant and leaf disc assays. Resistance to *F. occidentalis* was only partially consistent among the three sites in the Netherlands. The most susceptible accessions were consistently susceptible, but which accession was the most resistant differed among sites. In Asia, one *C. chinense* accession was particularly resistant to *S. dorsalis* and *T. palmi*, but this was not the most resistant accession to *F. occidentalis*. Overall, resistance to *F. occide*ntalis correlated with *S. dorsalis* but not with *T. palmi* resistance in the *C. annuum* accessions. Damage inflicted on leaf discs reflected damage on the whole plant level. Our study showed that identifying broad spectrum resistance to thrips in *Capsicum* may prove to be challenging. Breeding programmes should focus on developing cultivars suitable for growing in defined geographic regions with specific thrips species and abiotic conditions.

KEYWORDS
crop breeding, *Frankliniella occidentalis*, insect resistance, pepper, *Scirtothrips dorsalis*, *Thrips palmi*

1 | INTRODUCTION

Capsicum is a genus in the nightshade family (Solanaceae) which contains several species that are commercially grown as hot and sweet peppers. Commonly cultivated species include *C. annuum*, chili and sweet peppers, and *C. chinense*, aromatic hot peppers. Hot and sweet peppers are among the most produced crops, with a total annual production of approximately 34.5 million tons worldwide (FAOSTAT; Data Productions Crops 2016). The majority of chillies and peppers are produced in Europe and Asia, 11.5% and 64.9%, respectively (FAOSTAT; Data Productions Crops 2016; http://www.fao.org/faostat/en/#data/QC/visualize). For several centuries, *Capsicum* species have been domesticated, which has inadvertently led to the loss of natural resistance to insects. Consequently, many insect species, including several thrips species, contribute to yield losses in this plant genus (Cannon, Matthews, & Collins, 2007; Ssemwogerere, Ochwo-Ssemakula, Kovach, Kyamanywa, & Karungi, 2013; Walsh, Maltby, Nolan, & Kay, 2012; Weintraub, 2007). Thrips are small sucking piercing insects whose feeding cause stunted plant growth, leaf deformation and scarring of fruits (Tommasini & Maini, 1995; Welter, Rosenheim, Johnson, Mau, & Gusukuma-Minuto, 1990). Indirectly, these small insects cause yield losses due to spread of viruses, most prominently tospoviruses (Riley, Joseph, Srinivasan, & Diffie, 2011; Rotenberg, Jacobson, Schneweis, & Whitfield, 2015; Whitfield, Ullman, & German, 2005). Controlling thrips is challenging due to the emergence of thrips populations that are resistant to insecticides (Wang et al., 2016). In addition, insecticides are not sufficiently effective in killing all thrips and form a threat to beneficial insects such as biocontrol agents and bees (Brandt, Gorenflo, Siede, Meixner, & Büchler, 2016; Dively, Embrey, Kamel, Hawthorne, & Pettis, 2015). Identifying sources of natural resistance to thrips in *Capsicum* species has therefore become a necessity.

Preferably, this resistance should be effective under different abiotic conditions and to several thrips species, so that cultivars can be grown in different geographic regions. Resistance to thrips in *Capsium* has been reported (Fery & Schalk, 1991; Maharijaya et al., 2011; Maris, Joosten, Goldbach, & Peters, 2004; Visschers, Peters, van de Vondervoort, Hoogveld, & van Dam, 2019), but it is unclear whether previously identified resistance as assessed in a specific environment holds under different conditions. Environmental conditions such as temperature and light are known to modulate plant–insect interactions (Wang, Bao, Zhu, & Hua, 2009; Zavala, Mazza, Dillon, Chludil, & Ballare, 2015). In addition, biotype (or genotype) of the insect species may also play a role. For example, variation in performance among thrips populations has been reported on cucumber (*Cucumis sativus*) (Kogel, Hoek, & Mollema, 1997).

Furthermore, resistance to thrips in *Capsicum* can be thrips species-specific. In our previous work, we showed that resistance to *Frankliniella occidentalis* and *Thrips tabaci* was not correlated (Visschers et al., 2019). The work by Maharijaya et al. (2011), with a smaller test panel of 32 lines, showed that resistance to *F. occidentalis* was positively correlated with resistance to *Thrips parvispinus*. Partial species-specific resistance has also been shown in barrel clover (*Medicago truncalata*) in relation to aphid resistance. One of the tested accessions provided resistance to three aphid species, but was susceptible to two other aphid species (Gao, Horbury, Nair, Singh, & Edwards, 2007).

Other thrips species that commonly occur on *Capsicum* include *Thrips palmi* and *Scirtothrips dorsalis*. These thrips species are common in the tropical to subtropical regions and are quarantine organisms in the EU (EPPO/CABI, 1998; Vierbergen & van der Gaag, 2009). *Thrips palmi* is mostly found on leaves and to a lesser extent on the flowers (Rosenheim, Welter, Johnson, Mau, & Gusukuma-Minuto, 1990). Similar to *F. occidentalis* feeding, *T. palmi* feeding results in scarring and deformation of the leaves. At high densities it causes retarded plant growth (Kawai, 1986). *Thrips palmi* functions as a vector for economically important tospoviruses, including groundnut bud necrosis virus (Daimei et al., 2017) and watermelon silver mottle tospovirus (Chen, Tseng, & Tsai, 2014). *Scirtothrips dorsalis* is an important pest in India (Mound & Palmer, 2009). This thrips species can have devastating effects on the plant, eventually leading to its death. In pepper, *S. dorsalis* feeding results in "Chili leaf curl" (Sanap & Nawale, 1987) and at high infestation rates plant damage can be similar as that caused by broadmites (Kumar, Kakkar, McKenzie, Seal, & Osborne, 2012). When screening for general thrips resistance, it is useful to also take these thrips species in consideration.

Previously, we screened 40 *Capsicum* accessions for resistance to two thrips species using leaf disc assays (Visschers et al., 2019). This resulted in the identification of 11 accessions that were either relatively resistant or susceptible to *F. occidentalis* (Macel et al., 2019). Although leaf disc screening methods are widely accepted and used for pest resistance screening (van Rijn, Mollema, & Steenhuis-Broers, 1995; Thoen et al., 2016) and Maharijaya et al. (2011) convincingly showed the correlation between the detached leaf assay, leaf discs and whole plant damage, the question remains whether screening approaches using leaf discs reliably predicts resistance at the whole plant level for our set of *Capsicum* accessions.

Here, we tested (a) whether thrips resistance was robust in different environments with different thrips species/populations, (b) whether resistance in leaf discs assays reflects resistance at the whole plant level. To achieve these aims, 11 *Capsicum* accessions selected from a set of 40 accessions (*C. annuum* and *C. chinense*, Macel et al., 2019; Visschers et al., 2019, Figure 1) were screened for resistance to *F. occidentalis* at three different greenhouse locations in the Netherlands.

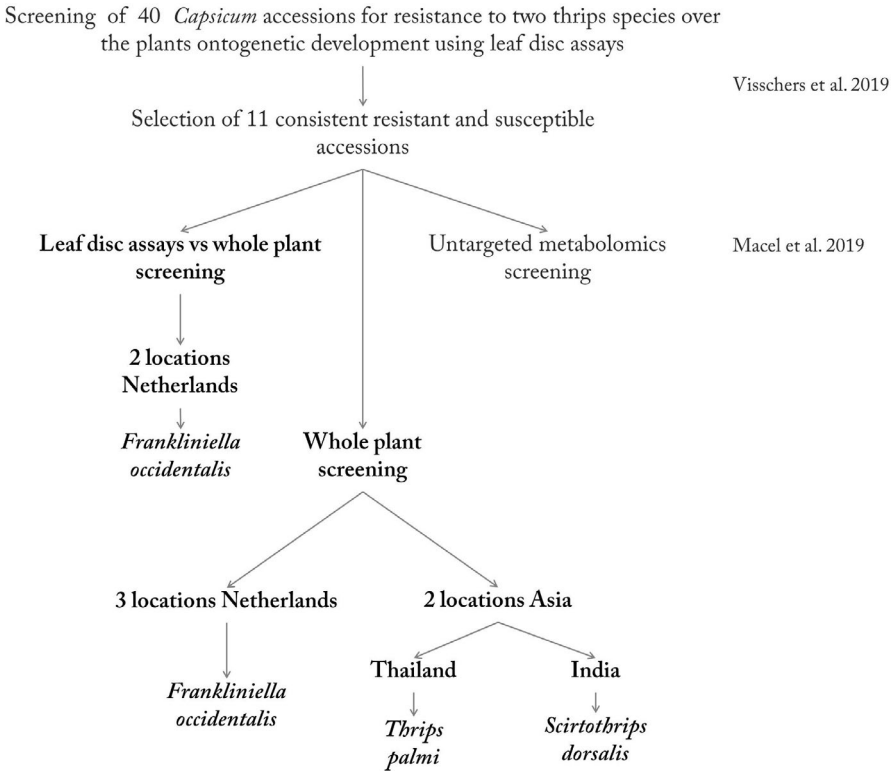

FIGURE 1 Schematic overview of previous studies conducted on resistance to thrips in *Capsicum* (Macel et al., 2019; Visschers et al., 2019) that formed the foundation of the experiments reported here (displayed in bold)

The 11 accessions were also screened for resistance to *T. palmi* and *S. dorsalis* at two locations in Asia. Combined, this resulted in a unique analysis of thrips resistance in *Capsicum* at five different locations.

2 | MATERIALS AND METHODS

2.1 | Plant material

We used two *Capsicum* species, *C. annuum* and *C. chinense* (Table S1) comprising 11 accessions that were selected from a thrips resistance screening of 40 accessions (Visschers et al., 2019). The eleven accessions were selected for being either among the most (5) or least (6) resistant (Macel et al., 2019). An overview of the experiments conducted is presented in Figure 1. Original seeds were obtained from the Centre for Genetic Resources (CGN), Wageningen University and Research Centre, the Netherlands (http://cgngenis.wur.nl/). Site 1 used seeds directly obtained from the CGN, after which seeds were multiplied from the original material for use at sites 2 and 3 and in Asia. Seeds of spreader plants *C. annuum*, accession "Super hot", marigold (*Tagetes erecta* L.), cowpea (*Vigna unguiculata*) and yard-long bean (*Vigna unguiculata* subsp. sesquipedalis), were obtained from East-West Seed.

2.2 | Experiment 1: whole plant screening experiment at 3 sites in the Netherlands

Experiments were conducted at De Lier and at two different locations in Enkhuizen, The Netherlands. Experiments were performed from August until October 2017 and from May until July 2018.

2.2.1 | Site 1

For the experiment on site 1, plants were exposed to a natural thrips infestation. The test plants were grown in soil in an unheated greenhouse, without additional lighting near Enkhuizen (52°43′25.738″ N, 5°16′54.987″ E), The Netherlands. Prior to infestation, accessions were sown and germinated in a clean nursery on rockwool under optimal conditions (Temperature 18/22°C, 16/8 hr light regime, supplementation to 6,000 Lux using 600 W Son-T lights). Four-week-old seedlings were transplanted to soil in the trial greenhouse, in a randomized block design (n = 9 plants per accession). At every fourth position, an additional highly susceptible *Capsicum* control plant was planted. Four weeks prior to the start of the trial, *F. occidentalis*, reared on runner bean (*Phaseolus coccineus*) under acclimatized conditions in plastic containers, were used to inoculate flowering yellow mustard (*Brassica nigra*) plants at the test site. Mustard plants were placed throughout the greenhouse compartment to allow the build-up of a solid thrips population and to ensure an even infection. At the start of the trial, when the *Capsicum* seedlings were transplanted, the thrips-infested mustard plants were cut down. One week after transplanting, thrips damage on the *Capsicum* plants was evaluated for the first time, following standard procedures by in-house experts. This was followed by weekly scoring over a 5-week period using a relative scale from 1 (susceptible; feeding damage throughout the plant, heavy damage oldest leaves, leaf drop, heavy growth deformation of young leaves) increasing to 9 with damage symptoms diminishing (resistant; no visible symptoms, even on oldest leaves).

2.2.2 | Site 2

At site 2, whole plant experiments were conducted in a greenhouse with gauze-sealed roof windows in Enkhuizen, The Netherlands (52°42′3.676″ N, 5°6′12.243″ E). Temperatures were set to 24/24°C and light was supplemented when below 400 W/m^2 using 10,000 lux Son-T lamps. Seeds were germinated in sowing trays with fine peat soil. After 2 weeks, seedlings were transplanted to pots (7 × 7 × 8 cm) and placed in the greenhouse (n = 31–48 plants per accession). Plants were randomly placed in two large 4 × 1 × 1 m gauze cages with a susceptible *C. annuum* accession at the borders. All plants were infected with 10,000 adult *F. occidentalis* in total, 4 weeks after sowing. Plants were then scored on silvering damage 5 weeks after infestation using a relative scale from 1 (susceptible, very heavy silvering, large part of the leaf damaged, leaf drop and heavy growth deformation of young leaves) increasing to 9 with damage symptoms diminishing (resistant, no silvering damage, no leaf deformation) following standard procedures by in-house experts. The thrips population used to infest the test plants was reared on garden beans (*Phaseolus vulgaris*).

2.2.3 | Site 3

Experiments were performed in a greenhouse with gauze-sealed roof windows in De Lier, The Netherlands (51°58′23.17″ N, 4°15′22.301″ E). Temperature was set to 23°C, and light intensity was 180–200 µmol m^{-2} s^{-1} PPFD at the plant growing level. Seeds were germinated in sowing trays with a 1:1 mixture of sand and potting soil. After 2 weeks, seedlings were transplanted to pots (0.7 L) and placed in the greenhouse (n = 8–30 plants per accessions). Test entries were distributed randomly in groups of five plants per accession over three tables. Plants were infected with *F. occidentalis* 3 weeks after sowing, by shaking thrips infected lettuce leaves over the plants. Two and three weeks after thrips infection, plants were scored using a scale from 0 (resistant, no visible symptoms of thrips damage, even on oldest leaves) via 2 (intermediate resistant, showing thrips damage symptoms on old leaves and little damage on young leaves) up to 4 (susceptible; very heavy silvering, large part of the leaf damaged, leaf drop and heavy growth deformation of young leaves) following standard procedures by in-house experts. Thrips populations used for infestation were maintained on lettuce (*Lactuca sativa*) plants. RU08 was not screened at this location due to low germination rates.

2.3 | Experiment 2: whole plant screening experiments at two sites in Asia

2.3.1 | Screening experiment with *Thrips palmi* in Thailand

Experiments in Thailand were conducted at an East-West Seed station in the Song PeNong District, Suphanburi, Thailand (14°12′19.047″ N, 99°52′18.8″ E). Experiments were performed from the end of December 2016 until mid-April 2017 in a plastic greenhouse (6 × 20 m). The greenhouse contained three tents made with crop cover cloth (N. White, UV stable, 17 g m^2, CTM Agro Textiles). Average morning and afternoon temperatures were recorded at 31°C and 42°C, respectively. Each tent contained two rows of test entries and two rows of susceptible hybrid Super Hot *C. annuum* flanking the test entries.

Insect rearing

Thrips palmi was reared on okra pods (*Abelmoschus esculentus*) and flowers of the susceptible hybrid Super Hot *C. annuum*, in plastic boxes and kept in an incubation room with a temperature set to 35–25°C (day/night). Next, Super Hot *C. annuum* and eggplant (*Solanum melongena*) seedlings were inoculated with the thrips in a nethouse with day temperatures between 35 and 40°C. Rearing of the thrips was started 2 weeks after sowing of the spreader plants.

Plant growth

Seeds were germinated in sowing trays with a mixture of ground, peat moss and coir dust (3:1:1). Seeds of test entries were sown 3 weeks after sowing of the spreader plants. After the first two leaves had emerged, the seedlings were transferred to pots (diameter 13.5 × 12 cm) filled with the same potting soil as used for germination. Spreader plants were placed in the greenhouse 6 weeks after sowing and inoculated with *T. palmi* 1 day thereafter. Test entries were placed in the greenhouse 3 weeks later. Germination rates of accessions RU32, RU27 and RU21 were low and a second batch of seeds was sown 5 weeks after spreader plants were sown. The plants of this batch were placed in the greenhouse, 5 weeks after sowing and were randomly placed among the rest of the test entries.

Data collection

Data collection started 2 weeks after test entries were placed in the greenhouse. During a 5-week period, test plants were ranked for thrips damage weekly using a scale from 1 (severe damage, stunted growth, deformed leaves) to 6 (no damage) (Figure S1, n = 21–36 plants per accession). In the first, third and fifth week of data collection, the abaxial side of a young apical leaf and middle leaf from each plant was photographed with a Nikon D90 equipped with an AF-S NIKKOR 18–105 mm 1:3.5–5.6 G ED lens (n = 7–20 plants per accession). We selected leaves that were representative for whole plant damage. In the second and fourth week of data collection, the presence of *T. palmi* was confirmed and presence of other thrips species was excluded. Three flowers of one plant per accession, including spreader plants, were collected. Thrips present in the flowers were placed in a KOH solution (30.8 g/L). After 24 hr, the thrips were flushed in water and prepared with mounting medium (100 g chloral hydrate, 60 g glycerine, 60 g gum arabic and 100 ml distilled water). Mitoc BA210E microscopes were used for determination of the thrips species. The following characteristics of *T. palmi* were used for identification: antenna with seven segments, on the antenna segments III and IV forked sense cones, the first vein of the forewing with three setae with gaps in distal half and the head with two pairs

of ocellar setae (pair II and III) (https://keys.lucidcentral.org/) (Figure S2). Only adult individuals were used for the identification. All thrips collected were identified as *T. palmi*.

2.3.2 | Screening experiment with *Scirtothrips dorsalis* in India

Experiments were conducted at East-West Seed's Mulani farm in Taluka-Paithan, Aurangabad, Maharasthra, India (19°47′2.208′′ N, 75°12′59.673′′ E), from February 2017 until the end of May 2017. Experiments were performed in a polyhouse with cooling system and exhaust fan (Nikhil Agrow Tech, Hyderabad, 6 × 20 m) with temperature set to 30/25°C (day/night). Due to frequent power failures, temperatures peaked to 51.2°C. The polyhouse was divided in three tents build with galvanized iron pipes and covered with crop cover cloth (N. White, UV stable, 17 g m^2, CTM Agro Textiles). Each tent contained test entries alongside the spreader plants *C. annuum*, accession "Super hot", marigold (*Tagetes erecta* L.), cowpea (*Vigna unguiculata*) and yard-long bean (*Vigna unguiculata* subsp. *sesquipedalis*).

Insect rearing

Scirtothrips dorsalis was collected from the field and reared in plastic Tupperware® jars (2 L) on aubergine (*Solanum melongena*) and yard-long bean (*Vignaunguiculata* subsp. *sesquipedalis*) fruits. Rearing jars were equipped with a 5 × 6 cm ventilation hole covered with crop cover cloth (N. White, UV stable, 17 g m^2, CTM Agro Textiles). The thrips were reared in jars during a 5-week period before they were used to inoculate the spreader plants.

Plant growth

Seeds of spreader plants and test entries were sown in a sowing tray with coco peat soil and placed in the polyhouse. Seeds of test entries were sown 4 weeks after sowing of spreader plants. The seedlings were watered daily and sprayed with acephate (0.5 mg/L) once a week. Seedlings were transferred to pots (diameter 22.86 × 27.94 inch) with a soil mixture (loamy soil 35%, black soil 20%, farmyard manure 20%, coco peat 20% and sand 5%), after the first two leaves had emerged. The plants were watered when needed, and fertilizer was added once a week (N:19, P:19, K:19; Nitrophoska and calcium nitrate: 1 g with potassium nitrate: 1 g). Spreader plants were divided over the three tents 8 weeks after seed sowing, test entries were placed 2 weeks later in the tents. Spreader plants were inoculated with *S. dorsalis* 1 day after they were placed in the greenhouse.

Data collection

Data collection was started 2 weeks after placement of the test entries in the polyhouse. During a 5-week period, plants were ranked for thrips damage weekly, using a scale from 1 (severe damage, stunted growth, deformed leaves, shed leaves) to 6 (no damage) (Figure S3, n = 4–25). In the 3rd week of data collection, thrips from each compartment were collected from the plants in 50 ml plastic tubes. In the laboratory, thrips were placed for 4 hr in a potassium hydroxide solution (154 g per 50 ml water) and afterwards placed in water. The thrips were prepared on microscopic slides with Hoyer's medium (chloral hydrate 100 g, glycerine 60 g, gum Arabic 60 g, distilled water 100 ml). Thrips species were identified under a microscope (Motic BA210E). Characteristics of *S. dorsalis* were identified: the yellow colouring, an 8-segmented antenna with a forked sensorium on segments 3 and 4, forewings with three setae on the distal half on the first vein and two widely spaced setae on the second vein, three pairs of ocellar setae, and two pairs of major postocular setae (https://keys.lucidcentral.org/) (Figure S4). All thrips were identified as *S. dorsalis*, and only one thrips was identified as *T. palmi*. In the 3rd week of data collection, the abaxial side of a middle leaf from the vertical axis of the plant was photographed with a Nikon D90 and AF-S NIKKOR 18–105 mm 1:3.5–5.6 G ED lens (n = 4–12 plants per accession). A black cloth served as black background to provide sufficient contrast with the leaves. Selected leaves were representative for whole plant damage.

2.3.3 | Image analysis

Image processing and quantification of feeding damage on leaves of the experiments in Asia was performed using Ilastik version 1.1.3 and ImageJ Fiji version 1.50i/Java 1.6.0_24 (64-bit) according to the protocol by Visschers, I, G, S, Dam van, N., M, and Peters, J., L. (2018b). Briefly, Ilastik was trained using four leaves per accession to recognize damage based on colour/intensity, colour gradient and texture at the level of 1 pixel. Three segments were identified: thrips damage, undamaged leaf area and the background. Training of the programme was continued until the three segments could be sufficient identified by the program. After training, images were converted to simple segmentations of the original image in black (thrips damage), grey (leaf disc) and white (background). In ImageJ Fiji, thrips damage and leaf area were calculated using the threshold function and analyse particles function. Calibration step I was not preformed, and in step K2, the "distance" and "known" distance in the macro were both set to 1. The percentage of thrips damage was then calculated using the following formula:

$$\%\ \text{damage leaf area} = \left(\frac{\#\ \text{pixels damaged leaf area}}{\#\ \text{pixels whole leaf area}}\right) \times 100$$

2.4 | Experiment 3: leaf disc assay at 2 sites in the Netherlands

At site 1 and 2, a separate batch of plants was grown for the leaf disc assay. Leaf samples were taken in the apical part of plants in the vegetative stage after 4 weeks of plant growth. Per accession 8–10 plants were used for this choice experiment. Leaf disc experiments were performed as described by (Visschers, van Dam, & Peters, 2018a). Using a cork borer, two leaf discs (1.5 cm diameter) were punched from each leaf, thereby avoiding the mid-vein. A leaf disc from each accession was placed on a drop of 1.5% slightly liquid agar with the abaxial side up in a Petri dish (9 cm diameter). Each Petri

dish (n = 24) thus contained 11 leaf discs (placed in a circle), each representing 1 of the 11 accessions. Twelve Petri dishes were inoculated with thrips. Per inoculated Petri dish, 22 L1/L2 *F. occidentalis* larvae, were placed in the middle of the dish using a small painting brush. For inoculation, the same thrips colonies were used as for the whole plant assays at each site in experiment 1. All Petri dishes were sealed with Parafilm and taken to the Radboud University, Nijmegen, The Netherlands, and placed in a climate cabinet (Economic Delux 432 L with TL lights; Snijders Labs) at 25°C and L16:D8 photoperiod. Petri dishes without thrips were directly sealed with Parafilm and used for correction during image analysis. After 48 hr, leaf discs were analysed and thrips feeding damage was determined using the protocol described by Visschers et al. (2018b).

2.5 | Statistical analysis

All statistical analyses were performed using R Version 1.0.153 (R Core Team, 2016). Damage scores of the whole plant screenings obtained in NL and Asia were standardized in classes, in such a way that low values uniformly represented low damage rates and high levels of resistance, and higher numbers represented severe damage and high levels of susceptibility (1–9 in case of site 1 and site 2, 0–4 in case of site 3 and 1–6 in case of experiments in Asia).

2.5.1 | Whole plant screening experiments in the Netherlands

At site 1, the effect of time (week) after infestation on thrips damage scores in the whole plant experiment was analysed using the non-parametric Kruskal–Wallis rank sum tests. At site 3, this effect was analysed with a Mann–Whitney *U* test. At site two, resistance levels were only measured once during the experimental period. To test the overall effect of *Capsicum* accession on damage scores, at site 1 and 3 damage scores were averaged over the experimental period. Thereby an average resistance measure could be obtained over the whole experimental period. At all three locations, the effect of accession on damage scores was assessed with a Kruskal–Wallis rank sum test. Post hoc pairwise differences between accessions in damage scores were analysed with Mann–Whitney *U* tests with false discovery rate (FDR) correction. To compare the screening results of *F. occidentalis* at the three sites, ranks were assigned to each accession for each site separately. The ranks were based on average whole plant thrips damage scores over the whole experimental period. The most resistant accessions received a 1, while the most susceptible accession received an 11. Correlations of resistance ranks between test sites were analysed using Spearman correlations.

2.5.2 | Whole plant screening experiments in Asia

The effects of time (week) after infestation on thrips damage scores was analysed using the non-parametric Kruskal–Wallis rank sum tests. Next, whole plant damage scores were averaged over the experimental period and the effect of accessions on damage scores was assessed with a Kruskal–Wallis test. Post hoc pairwise differences between accessions in thrips damage scores were analysed with Mann–Whitney *U* test with (FDR) correction. Significant effects were reported with alpha set to .004. A similar procedure was followed for damage percentage on selected leaves.

2.5.3 | Resistance ranking among thrips species

To compare the screening results of the different thrips species, a similar method was used as described for the comparison among test sites in the Netherlands. For the thrips species comparison, *F. occidentalis* ranks were based on overall average damage scores of all three tested sites. *S. dorsalis* and *T. palmi* ranks were based on average plant damage scores in Thailand and India, respectively. This comparison between thrips species was analysed separately for each *Capsicum* species, since our data indicated that each *Capsicum* species possibly possesses different thrips species-specific resistance mechanisms. Correlations of resistance ranks between thrips species were analysed using Spearman correlations.

2.5.4 | Leaf disc assay in the Netherlands

Thrips leaf disc choice assay data, that is percentage of damage per leaf disc relative to the total amount of damage per Petri dish, were analysed with a Friedman ANOVA for dependent data for site 1 and 2. Post hoc pairwise differences in relative damage per leaf disc of the choice assays were analysed with paired Wilcoxon signed rank test with (FDR) correction. To compare the screening results the leaf disc assay and the whole plant assay in the Netherlands, ranks were assigned to each accession for each screening method separately. Ranks were based on average whole plant thrips damage scores and relative damage fraction on leaf discs per accession. Ranks were assigned using a similar method as for the comparison between test sites. Correlations of resistance ranks between test methods were analysed using Spearman correlations.

3 | RESULTS

3.1 | Whole plant screening with *Frankliniella occidentalis* at three locations in the Netherlands

Resistance to *F. occidentalis* was determined at three different locations in the Netherlands. Whole plant damage scores were monitored over a period of several weeks at site 1 and 3. At site 2, damage scores were determined at one single time point. The damage scores at site 1 changed significantly over the 5 weeks of data collection in most of the accessions, except for RU06, RU32 which were consistently resistant over time and RU08 which was consistently susceptible (Table S2). At site 3, the damage scores differed only in accession RU32, this accession became slightly more susceptible over time (Mann–Whitney, W = 13.5, p < .001, Table S3).

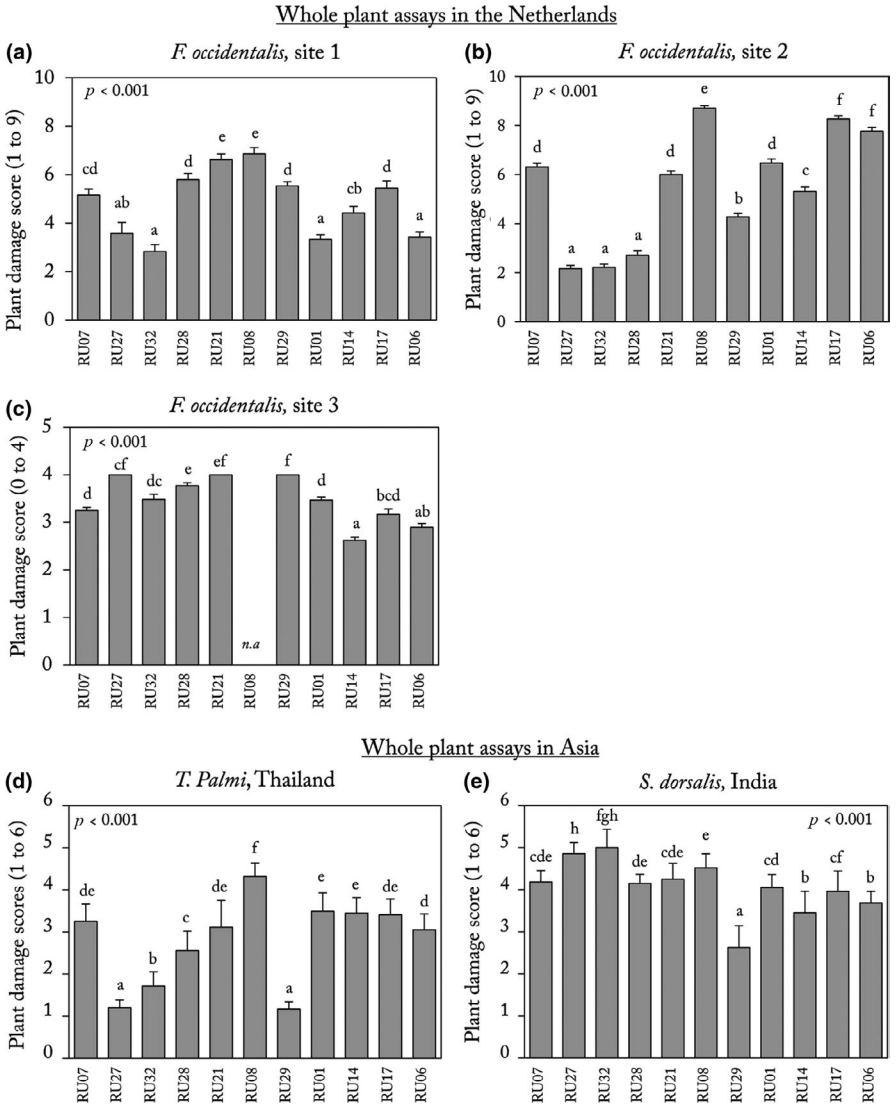

FIGURE 2 Resistance screening results of 11 *Capsicum* accessions with *Frankliniella occidentalis* (a–c), *Thrips palmi* (d) and *Scirtothrips dorsalis* (e). (a–c) Mean (±*SE*) damage scores of whole plant screening at three sites in the Netherlands (1 = little damage, 9 = severe damage for site 1 and 2, 0 = little damage, 4 = severe damage for site 3), *n* = 8–9 plants per accession for site 1, *n* = 31–48 for site 2 and *n* = 8–31 for site 3. Accession RU08 was not tested at site 3 due to low germination rates (n.a.). (d and e) Mean (±*SE*) damage scores of whole plant screening at two sites in Asia (1 = little damage, 6 = severe damage), *n* = 7–19 plants per accession for *T. palmi* and *n* = 4–25 for *S. dorsalis*. *p*-values of overall Kruskal–Wallis are given in each panel. Different letters indicate a significant difference between accessions ($p < .004$, Mann–Whitney *U* tests)

Whole plant thrips damage scores differed significantly among the *Capsicum* accessions at all three test locations (Kruskal–Wallis, site 1: $\chi^2_{10} = 175.5$, $p < .001$, site 2: $\chi^2_{10} = 349.9$, $p < .001$ and site 3: $\chi^2_{10} = 208.9$, $p < .001$, Figure 2a–c). Resistance ranking of *F. occidentalis* among the three sites revealed that screening results differed among these locations (Figure 3a). For example, accession RU32, resistant at both site 1 (rank 1) and site 2 (rank 2), was identified as a very susceptible accession at site 3 (rank 9) (Figure 3a). Despite these differences among the sites, accession RU27 could be identified as resistant at all three locations (rank 4, 1 and 3 at site 1, 2 and 3, respectively). RU08, although not tested at site 3, was the most susceptible accession at both other locations (rank 11).

3.2 | Whole plant screening with *Thrips palmi* in Thailand

Thrips palmi screening experiments were conducted at East-West Seed, Thailand. First we analysed whether *T. palmi* damage scores and damage percentages of selected leaves changed over the 5 weeks of data collection. In all accessions, whole plant damage scores and damage percentage on leaves remained constant over the experimental period (Table S4 and S5). Accessions significantly differed in thrips whole plant damage scores and damage percentages on the leaves (Kruskal–Wallis, damage score: $\chi^2_{10} = 447.4$, $p < .001$, damage percentage: $\chi^2_{10} = 250.6$, $p < .001$, Figure 2d and Figure S5a). Accessions RU29 and RU27 could be identified as highly resistant, while accession RU08 was found to be susceptible (mean whole plant damage scores: 1.2, 1.2 and 4.3, respectively; mean percentage of leaf damage: 0.2, 0.2 and 13.3, respectively). Interestingly, the four accessions most resistant to *T. palmi* all belonged to the species *C. chinense*. Correlation analyses of whole plant damage scores and leaf damage percentage revealed a significant positive correlation between these two damage measures (Spearman correlation, $p = .006$, $\rho(10) = .79$).

3.3 | Whole plant screening with *Scirtothrips dorsalis* in India

Scirtothrips dorsalis screening experiments were conducted in India. Thrips pressure was extremely high during the entire experimental

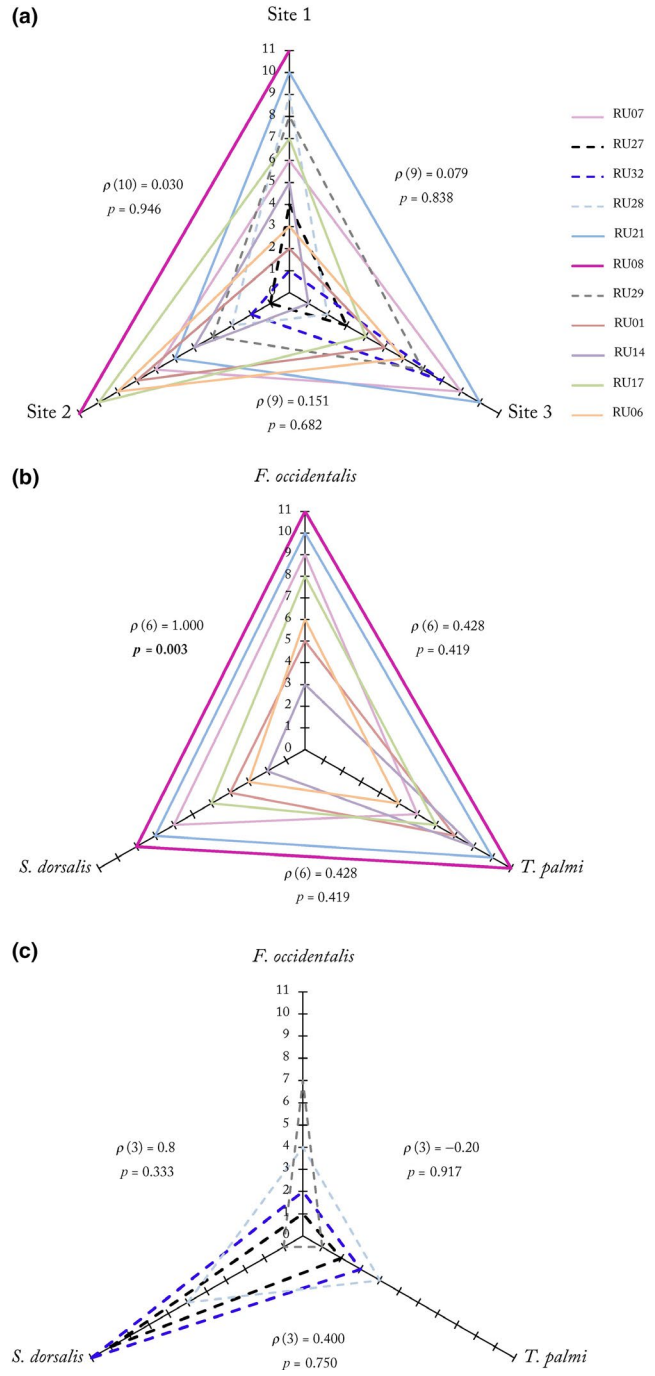

FIGURE 3 Three-dimensional resistance ranking of 11 *Capsicum* accessions. (a) *Frankliniella occidentalis* resistance ranking based on whole plant damage scores of the same accessions tested at three different sites in the Netherlands. Accession RU08 was not tested at site 3 due to low germination rates. (b and c) Resistance ranking based on whole plant thrips damage scores of the *C. annuum* (b) and *C. chinense* (c) accessions tested with *Frankliniella occidentalis*, *Scirtothrips dorsalis* and *Thrips palmi*. Rank 1 = low damage levels (resistant); rank 11 = high damage levels (susceptible). Different formatting of lines represents the different *Capsicum* species. *p*-values and rho (ρ) of Spearman correlation of thrips ranks between test sites (a) and thrips species (b and c) are given in the graphs

period. Consequently the damage scores increased strongly over the 5 weeks of data collection (Table S6), except for accession RU32 where damage was severe from the beginning. In the fifth week of data collection, damage scores of all the accessions increased to the maximum of 6 (Table S6). For this reason, only the scores obtained during the first 3 weeks of data collection were averaged to identify differences among the accessions. Damage scores differed significantly among accessions (Kruskal–Wallis, $\chi^2_{10} = 183.22$, $p < .001$, Figure 2e). Similar results were obtained for leaf damage percentage (Kruskal–Wallis, $\chi^2_{10} = 35.1$, $p < .001$, Figure S5b). Accession RU29 again could be identified as one of the most resistant accessions (Figure 2e). Correlation analyses of whole plant damage scores and damage percentage on leaves, showed a significant positive correlation between these two damage measures (Spearman correlation, $p = .007$, $\rho(10) = .78$).

3.4 | Resistance ranking among three thrips species

All 11 *Capsicum* accessions were screened for resistance to *F. occidentalis*, *T. palmi* and *S. dorsalis*. Resistance rankings based on whole plant assays revealed that resistance to thrips is partially thrips species-specific (Figure 3b and c). Resistance ranks of *F. occidentalis* and *S. dorsalis* were significantly correlated for the *C. annuum* accessions (Spearman correlation, $p = .003$, $\rho(6) = 1$, Figure 3b). *Thrips palmi* ranks did neither correlate with *F. occidentalis* nor with *S. dorsalis* resistance rankings. For the *C. chinense* accessions, no significant correlation between any of the thrips species could be observed (Figure 3c). Accessions RU27 and RU32 were resistant to *F. occidentalis* and *T. palmi* (RU27; rank 1 and 2 and RU32; rank 2 and 3, respectively), but susceptible to *S. dorsalis* (RU27 rank 10 and RU32 rank 11) (Figure 3b). Interestingly, accession RU08 was susceptible to all three thrips species (rank 11, 11 and 9, respectively, for *F. occidentalis*, *T. palmi* and *S. dorsalis*, Figure 3b).

3.5 | Leaf disc assays versus whole plant screening

At site 1 and 2 in the Netherlands, resistance to *F. occidentalis* was also determined using leaf disc assays. The accessions differed significantly in relative damage on leaf discs (Friedman ANOVA, site 1: $\chi^2_{10} = 67.3$, $p < .001$, site 2: $\chi^2_{10} = 72.1$, $p < .001$, Figure 4a and b). Leaf disc assay results and damage scores obtained by whole plant screening were significantly correlated at both sites (Spearman correlation, site 1: $p = .014$, $\rho(10) = .74$ and site 2: $p = .048$, $\rho(10) = .62$, Figure 4a and b). The screenings at both sites showed that accession RU08 was among the most susceptible to *F. occidentalis* while RU32 was the most resistant.

4 | DISCUSSION

Our analyses of thrips resistance in *Capsium* spp at five different locations revealed that resistance to *F. occidentalis* was only partially consistent among local test sites in the Netherlands. Resistance in *Capsicum* was found to be partly thrips species-specific. The

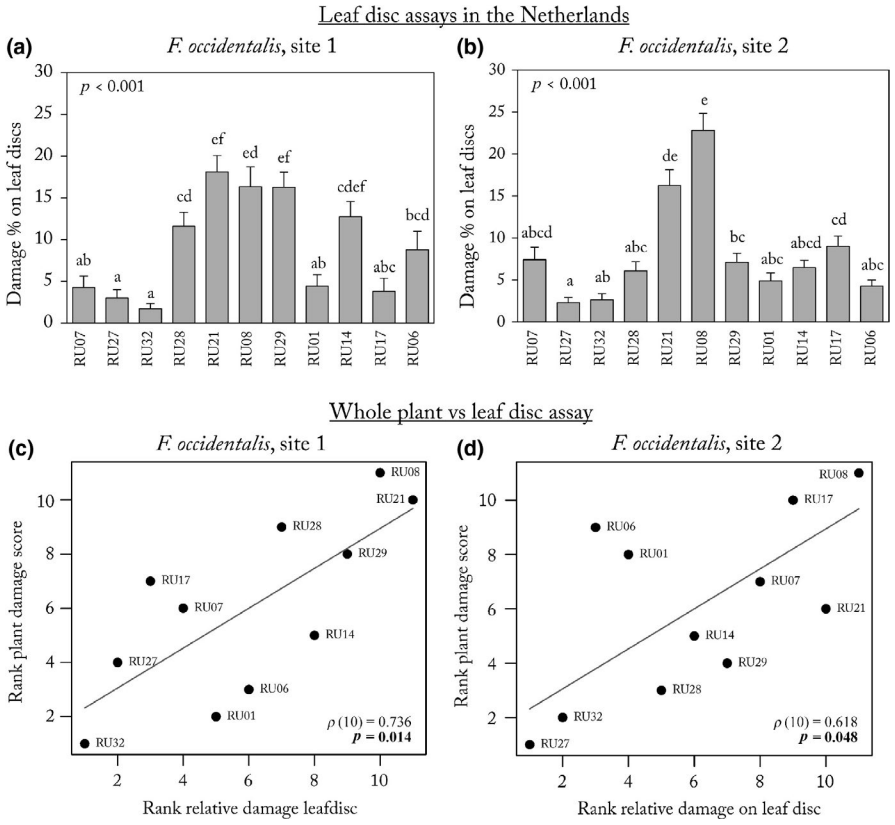

FIGURE 4 Resistance screening results of 11 *Capsicum* accessions with *Frankliniella occidentalis* at sites 1 (a and c) and site 2 (b and d) in the Netherlands. (a and b) Mean (±*SE*) damage percentage of leaf discs in choice assays, *n* = 12 Petri dishes per accession. *p*-values of overall Friedman ANOVA for dependent data are given in both panels. Different letters indicate significant differences between accessions ($p < .004$, Mann–Whitney *U* tests). (c and d) Rank correlation of *F. occidentalis* damage between whole plants assays (plant damage score) and leaf disc assays (relative damage on leaf discs). *p*-values and rho (ρ) of Spearman correlation are given in the graph

accession most resistant to *T. palmi* and *S. dorsalis* was not the most resistant accession to *F. occidentalis*. Resistance to *F. occidentalis* was significantly correlated to *S. dorsalis* but not to *T. palmi* in the *C. annuum* accessions. We further showed that damage inflicted to leaf discs reflects resistance measured on the whole plant level.

4.1 | Thrips resistance in different environments with different thrips species

In our study, we screened the same accessions for resistance to *F. occidentalis* at three different sites in the Netherlands. In most of the accessions, resistance to thrips differed among sites with the exception of accession RU27 (consistently resistant) and RU08 (consistently susceptible). These accessions have not been previously reported for being resistant and susceptible by Maharijaya et al. (2011), Maris, Joosten, Goldbach, and Peters (2003) or Fery and Schalk (1991). The differences in resistance among sites within accessions may be explained by different local thrips biotypes. Within several thrips species, the occurrence of biotypes and genetic differentiation of local populations has become evident. For example, in cucumber it has been shown that different *F. occidentalis* populations, originating from greenhouses in the Netherlands, Italy and New Zealand, showed significant differences in performance on the same cucumber (*Cucumis sativus*) genotype (Kogel et al., 1997). Even on a smaller geographic scale, differences in performance among thrips populations have become evident. Reproductive performance of *F. occidentalis* populations collected from different greenhouses in the Netherlands was significantly different (Mirnezhad, Schidlo, Klinkhamer, & Leiss, 2012). Amplified fragment length polymorphism (AFLP) analyses revealed that these populations showed clear genetic differentiation (Mirnezhad et al., 2012). Similarly, in *T. tabaci* there was genetic differentiation among 22 populations collected from different host plant species (Brunner, Chatzivassiliou, Katis, & Frey, 2004). Within this thrips species two biotypes, the "tabaci" and the "communis" type have been described, that differ in their efficiency of transmitting tomato spotted wilt virus (Chatzivassiliou, Peters, & Katis, 2002; Westmore, Poke, Allen, & Wilson, 2013; Zawirska, 1976). The occurrence of different local *F. occidentalis* biotypes may be a possible explanation for our observed differences in resistance levels within a single accession among sites. Our study underlines the importance of including different thrips populations or biotypes for identifying sources of broad spectrum resistance.

Another factor that might explain our observed differences in resistance levels among sites include environmental and seasonal variation in plant resistance to insects. The experiments at site 1 and 3 were both conducted during the summer, but in different years (2017 and 2018, respectively), while experiments at site 2 were conducted in the fall of 2017. Although all experiments were conducted in climate controlled greenhouses, the temperature and light conditions are mostly season dependent. These environmental factors are known to modulate plant–insect interactions (Escobar-Bravo et al., 2018; Wang et al., 2009; Zavala et al., 2015). Seasonal effects on insect resistance have been shown for example in *Barbarea vulgaris* ssp. *arcualata* accession (Agerbirk, Olsen, & Nielsen, 2001). During summer, an accession of this plant species was found to be resistant to the flea beetle *Phyllotreta nemorum*, but gradually lost

its resistance with the onset of fall which was related to changes in plant hormone levels (Agerbirk et al., 2001). In wild cabbage (*Brassica oleracea*), the concentrations of glucosinolates, which are secondary metabolites acting as chemical defences to insects, increased from summer to winter (Gols et al., 2018). Possibly seasonal variation may play a role in modulating resistance to thrips in *Capsicum*. This should be assessed by screening the accessions for resistance to *F. occidentalis* in the field throughout the seasons.

In our current study, we showed that in *C. annuum* resistance to *F. occidentalis* was strongly positively correlated with *S. dorsalis*, but not with *T. palmi*. This indicates that resistance might be partially thrips species-specific. Previous studies indicated that resistance to *F. occidentalis* was positively correlated with resistance to *T. parvispinus*, but not with resistance to *T. tabaci* (Maharijaya et al., 2011; Visschers et al., 2019). Interestingly, our study further showed that all *C. chinense* accessions were resistant to *T. palmi*. This suggests that within each *Capsicum* species resistance to thrips might be driven by different defence mechanisms. This hypothesis is supported by untargeted metabolomics analyses of the same accessions. Both *Capsicum* species possessed a unique set of metabolites that were correlated to resistance to *F. occidentalis* (Macel et al., 2019).

Although resistance to *T. palmi* and *S. dorsalis* were mostly not correlated, one *C. chinense* accession (RU29) could be pinpointed as highly resistant to these two thrips species in Asia, while susceptible to *F. occidentalis*. This accession was previously identified as susceptible by Maharijaya et al., 2011 and is also known for carrying resistance genes to tomato spotted wilt virus (Boiteux & de Ávila, 1994), a tospovirus that is vectored by thrips (Allen & Broadbent, 1986; Lemmetty & Lindqvist, 1993). However, this accession is known to be susceptible to whitefly (*Bemisia tabaci*) (Firdaus et al., 2011). Since thrips and whitefly have different feeding strategies, there may be possible trade-offs in resistance mechanisms between these cell sucking and phloem feeding insects, respectively. Therefore, accession RU29 is an excellent source of resistance to thrips in Asia. At the same time, it would be an ideal model plant for studying potential trade-offs in plant defence strategies to insects with contrasting feeding styles.

Differences in thrips species-specific resistance in *Capsicum* might also be explained by diversity in thrips effector proteins among thrips species. Upon feeding, thrips inject saliva into the plant tissue (Chisholm & Lewis, 1984). The effector proteins found in this saliva can trigger diverse immune responses or even suppress the immune responses in the plant, as has been shown for many other insects (Elzinga, De Vos, & Jander, 2014; Hogenhout & Bos, 2011). Thus, far, little is known about thrips and their effector proteins. Analysis of salivary glands of *F. occidentalis* by transcriptome analysis led to the identification of several genes that might play a role in detoxification and inhibition of plant defence responses (Stafford-Banks, Rotenberg, Johnson, Whitfield, & Ullman, 2014). Possibly, each thrips species or biotype might possess specific effector proteins that differ in their effects on plants. This can enable certain thrips species to successfully establish on cultivars and *Capsicum* species resistant to other thrips species. Further research on thrips effector proteins diversity among species and biotypes could therefore provide an important step in understanding the mechanisms of thrips species-specific resistance in *Capsicum*. Preferably, these experiments with different thrips species and biotypes should be conducted under fully controlled environmental conditions, for example in climate chambers, to further substantiate the findings in our current study. In addition, this would allow us to test whether thrips species-specific resistance is modulated by environmental conditions such as temperature regimes. Unfortunately, these highly controlled test using all four thrips species were not possible, because *T. palmi* and *S. dorsalis* are quarantine organisms in Europe.

4.2 | Whole plant versus leaf disc assays

Our results provide experimental evidence that leaf disc assays are a suitable method for screening resistance to thrips in *Capsicum* (Maharijaya et al., 2011; Visschers et al., 2018a). In longer-term whole plant screening assays, herbivore-induced defences may play a role when screening for resistance to insects (Dillon, Chludil, Reichelt, Mithöfer, & Zavala, 2018). In leaf discs assays, (volatile) compounds leaching from wounds may influence thrips damage scores. Nevertheless, our study showed that the resistance ranking of accessions was comparable between both methods, regardless of any induced responses that may occur. Leaf disc assays thus provide a reliable high-throughput method for screening for thrips resistance in *Capsicum*.

5 | CONCLUSIONS

Our study underscores that identifying broad spectrum resistance to thrips in *Capsicum* may be challenging. In some other plant species, broad spectrum resistance has been reported (Chen, Senthilkumar, et al., 2014; Senthilkumar, Cheng, & Yeh, 2010; Vosman et al., 2018). For example, in wild tomato (*Solanum galapagense*), the Wf-1 QTL region was linked to resistance to a diverse group of insects, including thrips (Vosman et al., 2018). In *Capsicum*, the few QTL mapping studies on thrips resistance focused only on *F. occidentalis* (Maharijaya et al., 2018, 2015). It is unclear whether the one currently identified QTL for *F. occidentalis* resistance applies to resistance to other thrips species, but our results suggest this is unlikely. In other plant–insect combinations, resistance mechanisms were also found to be highly insect species-specific, even on the level of the developmental stage of the insect (Hilder & Boulter, 1999; Lucatti et al., 2014; Soria & Mollema, 1995). Future development of genetic markers for thrips resistance in *Capsicum* should thus include additional important thrips species such as *T. palmi* and *S. dorsalis*, and additional biotypes of *F. occidentalis*. Due to the specificity of the resistance to different thrips, breeding programmes may have to focus on developing specialized cultivars suitable for growing in defined geographic regions with specific abiotic conditions and in the presence of the locally abundant thrips species.

ACKNOWLEDGEMENTS

This work was supported by the NWO Domain Applied and Engineering Sciences (AES) and is part of the Green defence Against Pest (GAP) programme, project 13552. We thank East-West Seed for facilitating the experiments conducted in Asia, and Rijk Zwaan, Syngenta and Enza Zaden for facilitating experiments in the Netherlands. We especially like to express our gratitude to Gopal Kulkarni (East-West Seed) and Sushil Kumar (Rijk Zwaan) for their support while conducting experiments in Asia. Nicole M. van Dam gratefully acknowledges the support of the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig funded by the German Research Foundation (FZT 118).

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHORS' CONTRIBUTIONS

IGSV conceived research, conducted experiments, analysed data and wrote the manuscript. JLP wrote the manuscript. LLHT and EE conducted experiments and analysed data. JBF, CHB and MS contributed material. PMB, ZvH, GAG and JB conducted experiments. NMvD secured funding and wrote the manuscript. MM conceived research, conducted experiments and wrote the manuscript.

DATA AVAILABILITY

Data supporting this manuscript will be made available from the 22th of November 2019 onwards.

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SUPPORTING INFORMATION

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How to cite this article: Visschers IGS, Peters JL, Timmermans LLH, et al. Resistance to three thrips species in *Capsicum* spp. depends on site conditions and geographic regions. *J Appl Entomol.* 2019;00:1–13. https://doi.org/10.1111/jen.12677