# Location of resistance factors in the leaves of potato and wild tuber-bearing *Solanum* species to the aphid *Myzus persicae*

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**Abstract**

Analysis of electrically recorded feeding behaviour of aphids was combined with colony-development tests to search for sources of resistance to *Myzus persicae* (Sulzer) (Homoptera: Aphididae) in tuber-bearing *Solanum* species (Solanaceae), aiming at a reduction of potato leaf roll virus (PLRV) transmission. Twenty genotypes, originating from 14 gene bank accessions, representing 13 wild tuber-bearing *Solanum* spp., three *Solanum tuberosum* L. (potato) cultivars, and one *S. tuberosum* breeding line, were selected. Colony-development tests were carried out in no-choice experiments by placing adult aphids on plants of each genotype and counting numbers of nymphs and adults on young plants after 8 and 15 days, and on flowering plants after 14 and 30 days. Large differences were observed among genotypes: some developed small colonies and others developed large ones. Also, in a few genotypes, resistance in mature plants was different for leaves of different ages; young leaves were resistant to aphids whereas old senescent leaves were susceptible. The electrical penetration graph (DC-EPG system) technique was used to study aphid feeding behaviour on each *Solanum* genotype for 6 h. Electrical penetration graph (EPG) results also showed large differences among the genotypes, indicating resistance at the leaf surface and at three different levels of plant tissue (epidermis, mesophyll, and phloem). Therefore, it was concluded that different mechanisms of resistance to *M. persicae* exist among the genotypes analysed. EPGs recorded from aphids on *Solanum berthaultii* Hawkes and *Solanum tarijense* Hawkes with and without glandular trichomes showed that strong surface resistance can bias EPG parameters associated with resistance located in deeper tissues. Experimental evidence is presented that the resistance to aphids in the genotypes with glandular trichomes strongly depends on these morphological structures.

## Introduction

With 235 recognized species, wild *Solanum* spp. (Solanaceae) represent a rich biological diversity, although only a few species gave rise to the cultivated potato (Hawkes, 1990). Until now, genes from at least 18 wild *Solanum* species have been incorporated into North American and European potato varieties. So far, no commercial potato varieties have been developed specifically for resistance to insects (Flanders et al., 1999). The green peach aphid, *Myzus persicae* (Sulzer) (Homoptera, Aphididae), has a worldwide distribution and causes damage principally indirectly by its ability to transmit plant viruses (Salazar, 1996). At least 54 viruses are known to infect potatoes (Brunt et al., 1996) and aphids are their most important vectors (Peters, 1987; Raman & Radcliffe, 1992; Jeffries, 1998). In potato crops, the percentage of plants infected with potato leaf roll virus (PLRV) strongly depends on the number of aphids that have previously visited PLRV-infected plants (Beekman, 1987). A low degree of PLRV

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infestation in seed potatoes normally poses no risk to commercial production when no green peach aphids are present (Flanders et al., 1999). Therefore, control of PLRV depends on effective control of green peach aphids (Radcliffe & Ragsdale, 2002). Growers depend on insecticides for suppression of *M. persicae* but selection pressure has resulted in several forms of resistance to these insecticides. Moreover, the negative environmental impacts of insecticides indicate that alternative control strategies and host plant resistance are needed.

Some wild *Solanum* species have effective resistance mechanisms against aphids. *Solanum berthaultii*, *Solanum polyadenium*, and *Solanum tarijense* have been studied extensively because of their resistance to aphids due to the presence of glandular trichomes (Gibson, 1971, 1974, 1976; Gibson & Turner, 1977; Tingley & Laubengayer, 1981). However, the glandular trichome-based resistance is genetically complex and it is strongly associated with poor agronomical characteristics (Kalazich & Plaisted, 1991; Bonierbale et al., 1994). Therefore, it has not yet been used successfully in breeding for aphid resistance.

More than 60 wild *Solanum* species have been reported to possess genotypes with resistance to *M. persicae* (Radcliffe & Lauer, 1968; Tingey & Sinden, 1982; Gibson & Pickett, 1983; Flanders et al., 1992, 1999; Novy et al., 2002). These species represent an important alternative source of aphid resistance, which can be exploited to enhance vector resistance in cultivated potato. Nothing is known about the underlying biochemical, physiological, and molecular mechanisms in these species. Aphids, as phloem-feeding insects, must insert their mouthparts into the plant (probing) in order to select a suitable host. Therefore, knowledge on probing behaviour of aphids is crucial for a better understanding of both aphid and virus control.

The electrical penetration graph (EPG) technique, introduced by McLean & Kinsey (1964) and further improved by Tjallingii (1978, 1985, 1988), is a robust tool to study plant penetration by the aphid's stylets. EPG signals have been correlated with aphid activities as well as with tissue locations of the stylet tips (Tjallingii, 1978, 1988; Kimmins & Tjallingii, 1985; Tjallingii & Hogen Esch, 1993). As such, EPG parameters can be used to identify the tissues containing the resistance factors (Tjallingii, 1995) and to infer the effects of the aphid-resistant plants on virus transmission (Prado & Tjallingii, 1994; Martín et al., 1997). In several studies EPGs have been used to obtain more information on the location of the resistance factors in host plant tissues (Givovich & Niemeyer, 1991; van Helden & Tjallingii, 1993; Cole, 1994; Gabrys et al., 1997; Klingler et al., 1998; Lei et al., 1999; Garzo et al., 2002; Klingler et al., 2005). The aim of the present study is to combine aphid bionomical data with EPG monitoring in order (1) to identify/confirm wild tuber-bearing *Solanum* genotypes resistant to *M. persicae* and (2) to obtain information on tissue location and the possible mechanism of resistance active in different *Solanum* genotypes that may play a role in PLRV transmission.

## Materials and methods

### Plants and aphids

Twenty *Solanum* genotypes were selected from the in vitro-culture collection maintained at the Laboratory of Plant Breeding (Wageningen University, Wageningen, The Netherlands). The original material was obtained from the Centre for Genetic Resources (CGN) (Wageningen, The Netherlands). Most genotypes selected belong to species of which some accessions previously have been reported to show some level of resistance to *M. persicae* (Radcliffe & Lauer, 1968; Hanneman & Bamberg, 1986). However, at the start of the evaluation process, we did not know whether the material selected possessed the reported resistance. The selected material included 13 wild tuber-bearing *Solanum* species, three *Solanum tuberosum* cultivars, and one breeding line of *S. tuberosum* (listed in Table 1). After some initial experiments, we selected the diploid breeding line *S. tuberosum* RH89-039-16 as the susceptible control. This genotype has frequently been used in our laboratory for interspecific crossings with wild *Solanum* spp. The propagation of plants was performed in vitro on Murashige and Skoog medium including vitamins, sucrose 3%, pH 5.8. After 2 weeks the plantlets were transferred to soil in a glasshouse at $22 \pm 2$ °C, about 70% r.h., and L16:D8 photoperiod.

In order to avoid a behavioural bias towards susceptible cultivated potato, we reared the aphids on radish (*Raphanus sativus* L.); hence the aphids could not adapt to any of the *Solanum* genotypes used. All aphids used in the experiments came from a newly established clone of *M. persicae* from a single virginoparous apterous individual from a colony maintained at the Laboratory of Entomology (Wageningen University). Colonies were reared in a climate chamber at $22 \pm 2$ °C, 30–40% r.h., and L16:D8 photoperiod. A new colony was started weekly and newly moulted adult aphids were collected later for the EPG tests.

### Aphid colony-development test

*Young plants.* For all genotypes (Table 1), resistance to *M. persicae* was tested in a no-choice experiment. Five recently moulted (1–3 days old) adult apterae of *M. persicae* were transferred to the first fully expanded leaf of one plant of each genotype (three plants per genotype, 1–2 weeks after transfer from agar to soil) enclosed in a Perspex cylinder, 130 mm high and 80 mm in diameter,

**Table 1** Aphid colony development test. *Solanum* species are ordered according to the number of aphids (adults and nymphs) per plant on day 15 in the aphid colony-development test on young plants. Values are means ± SEM of number of aphids (adults and nymphs)

| | | Young plants | | | | Flowering plants[3] | | |
|---|---|---|---|---|---|---|---|---|
| *Solanum* species | Identity number or cultivar name[1] | Adults on day 8 | Nymphs on day 8 | Adults on day 15 | Nymphs on day 15 | y | m | s |
| *S. okadae* Hawkes et Hjerting | 18108-3 | 29.7 ± 6.1 | 33.3 ± 4.1 | 29.3 ± 8.8 | 237 ± 81.2 | ++ | ++ | ++ |
| *S. tuberosum* L. | cultivar Eersteling | 4.7 ± 0.7 | 14.7 ± 2.7 | 21.0 ± 3.6 | 95.3 ± 27.8 | nd | nd | nd |
| *S. phureja* Juz et Buck. | 17667-1 | 1.0 ± 0.6 | 27.3 ± 3.7 | 9.7 ± 1.2 | 80.0 ± 10.8 | nd | nd | nd |
| *S. tuberosum* L. | RH89-039-16[2] | 0.7 ± 0.7 | 14.0 ± 1.0 | 11.0 ± 2.6 | 65.3 ± 16.2 | ++ | ++ | ++ |
| *S. hondelmannii* Hawkes et Hjert | 18182-2 | 1.3 ± 0.7 | 25.7 ± 8.8 | 10.3 ± 2.3 | 49.3 ± 30.7 | nd | nd | nd |
| *S. jamesii* Torr. | 18349-10 | 1.0 ± 0.6 | 13.7 ± 3.2 | 5.3 ± 2.3 | 31.0 ± 6.2 | nd | nd | nd |
| *S. berthaultii* Hawkes | 20644-6 | 7.7 ± 1.2 | 12.7 ± 4.8 | 5.0 ± 1.7 | 31.0 ± 5.2 | ++ | ++ | ++ |
| *S. bulbocastanum* Dun. | 17693-2 | 0.7 ± 0.7 | 11.7 ± 4.4 | 4.0 ± 1.7 | 13.3 ± 6.0 | nd | nd | nd |
| *S. jamesii* Torr. | 18349-1 | 1.0 ± 0.0 | 7.0 ± 2.1[4] | 2.3 ± 0.9 | 7.3 ± 3.2 | – | – | + |
| *S. tuberosum* L. | cultivar Mondial | 4.0 ± 1.2 | 3.0 ± 0.6[4] | 2.0 ± 1.2 | 7.3 ± 4.1 | ++ | ++ | ++ |
| *S. tuberosum* L. | cultivar Kardal | 0.7 ± 0.7 | 2.0 ± 1.2[4] | 1.0 ± 0.6 | 8.0 ± 4.0 | – | + | ++ |
| *S. spegazzinii* Bitt. | 17839-2 | 0 | 3.3 ± 1.2[4] | 2.0 ± 0.6 | 5.0 ± 0.6 | + | + | + |
| *S. capsicibaccatum* Cárd. | 18268-2 | 0 | 1.7 ± 0.3[4] | 0.7 ± 0.3[4] | 3.0 ± 1.0[4] | – | – | + |
| *S. capsicibaccatum* Cárd. | 18268-5 | 0 | 0.7 ± 0.3[4] | 0[4] | 1.3 ± 0.9[4] | nd | nd | nd |
| *S. multiinterruptum* Bitt. | 17829-2 | 0 | 0.3 ± 0.3[4] | 0[4] | 1.0 ± 1.0[4] | ++ | ++ | ++ |
| *S. cardiophyllum* Lindl. | 18326-1 | 0 | 3.0 ± 0.6[4] | 0[4] | 0.3 ± 0.3[4] | ++ | ++ | ++ |
| *S. berthaultii* Hawkes | 20650-3 | 0 | 0[4] | 0[4] | 0[4] | – | – | – |
| *S. stoloniferum* Schlechtd. | 17605-4 | 0 | 0[4] | 0[4] | 0[4] | – | + | ++ |
| *S. polyadenium* Greenm. | 17749-1 | 0.3 ± 0.3 | 1.7 ± 1.2[4] | 0[4] | 0[4] | + | – | + |
| *S. tarijense* Hawkes | 17861-8 | 0 | 0.3 ± 0.3[4] | 0[4] | 0[4] | – | – | – |

Dotted lines group species according to relative level of resistance.

[1]Identity numbers from Centre for Genetic Resources followed by the genotype in vitro collection number (e.g., -2) or cultivar name (cultivar).

[2]RH89-03916, breeding line chosen as susceptible control (diploid line used in interspecific crossings with wild *Solanum*).

[3]y, young leaves; m, mature leaves; s, senescent leaves, – no aphids found, + few aphids, ++ leaves covered by aphids and nymphs; nd, not determined (experiment not performed).

[4]Smaller number of aphids than the susceptible control *Solanum tuberosum* RH89-03916 (Mann–Whitney; $P<0.05$).

sealed with nylon mesh at the cylinder's top and on two lateral holes of 25 mm of diameter. The assays were conducted in a climate chamber at 22 ± 2 °C, 30–40% r.h., and L16:D8 photoperiod. The total numbers of aphids, adults, and nymphs were counted on days 8 and 15 of the experiment.

*Flowering plants.* Resistance to *M. persicae* was also tested in flowering plants, 5–8 weeks after transfer from agar to soil. Five adult apterae of *M. persicae* were transferred to the apical leaf of each plant. Four plants for each genotype were placed in cages (46 × 46 cm and 56 cm high) under glasshouse conditions (22 ± 2 °C, about 70% r.h., at L16:D8 photoperiod). After 14 and 30 days, plants were inspected for the presence and location of aphids. Three plant regions based on the maturity stage of the leaves were taken into account: (i) young apical, not fully expanded leaves, (ii) mature, fully expanded leaves, and (iii) senescent, yellowing leaves. The scoring was performed in a qualitative way (Table 1).

#### Electrical penetration graph monitoring of aphid probing behaviour on 20 *Solanum* genotypes

The DC-EPG technique (Tjallingii, 1985, 1988) was used to monitor probing of young adult and apterous aphids during 6 h. Four plants of a genotype were placed in a Faraday cage; probing and feeding behaviour of two aphids on each plant was recorded simultaneously during 6 h. Between 15 and 19 successful replicates were obtained per genotype. Each plant was used 20 days after being transferred to soil. Aphids were placed on the abaxial side of the third leaf from the apex, which was nearly fully

expanded. Before exposure to the plant, the aphid was attached to the electrode – a 2- to 3-cm-long gold wire (diameter 20 μm), conductively glued (water-based silver glue) to the dorsum – while immobilized by a vacuum suction device (van Helden & Tjallingii, 1993). The other end of the gold wire was attached to a 3-cm-long copper wire (diameter 0.2 mm) and connected to the input of the first stage amplifier with a 1 giga-ohm input resistance and 50× gain (Tjallingii, 1985, 1988). The plant electrode, a 2-mm-thick, 10-cm-long copper rod, was inserted in the soil of the potted plant and connected to the plant voltage output of the EPG device (Giga-4 or Giga-8, manufactured by Wageningen University). In addition to the plants, the aphids and the first stage amplifiers were set up in a Faraday cage. The recording was started immediately after aphid wiring, at about 20 °C and underconstant light in the laboratory, and about 1 h after collecting the aphids from the colony. Signals of eight plants, in two Faraday cages, were simultaneously acquired and recorded on a PC (one per setup) hard disk. Data acquisition and waveform analysis were mediated by PROBE 3.0 software (Laboratory of Entomology, Wageningen University) at 100 samples per second.

#### Electrical penetration graphs on *Solanum berthaultii* and *Solanum tarijense* with and without intact glandular trichomes

To test whether *S. tarijense* 17861-8 and *S. berthaultii* 20650-3, in addition to the strong surface resistance, posses another type of resistance, we recorded probing on *S. berthaultii* 20650-3 and *S. tarijense* after washing the leaves, which eliminated glandular trichome effects. The abaxial side of the third leaf from the apex of 20-day-old *S. berthaultii* 20650-3 and *S. tarijense* 17861-8 plants was mechanically wiped off using a cellulose cleaning tissue under running tap water. After this procedure, the leaf surface was inspected under a stereomicroscope to check that all glandular parts and secretions of the trichomes were removed. When the leaf surface was dry, after 30 min, probing behaviour of aphids was studied using the EPG technique as described above. Four plants per treatment were used. Two intact plants and two treated plants of the same genotype were placed together in a Faraday cage and EPGs of two aphids on each plant were recorded simultaneously during 6 h. Fifteen successful replicates were obtained per genotype. Intact plants of each genotype were used as control.

#### Electrical penetration graphs waveforms, waveform patterns, and parameters

The recorded EPGs were analysed by distinguishing the following waveforms or waveform patterns. Waveform C, stylet pathway phase, including four pooled pathway waveforms/activities, i.e., waveform A, epidermis first stylet contact; waveform B, intercellular sheath salivation; waveform C, stylet movements; and waveform pd (potential drop), an intracellular stylet puncture; waveform E, phloem phase, separated in waveform E1, sieve element salivation and waveform E2, phloem sap ingestion with concurrent salivation; waveform E1e, assumed watery salivation at extracellular voltage level; waveform F, derailed stylet mechanics (stylet penetration difficulties); and waveform G, active drinking of water from xylem elements (Tjallingii, 1990). Out of the 25 EPG parameters that we analysed, eight appeared to be most relevant for resistance to aphids in the analysed genotypes. These are as follows. (1) The time to the first probe, which represents the period between plant access (i.e., onset of the EPG recording) and the start of the first probe. A prolonged period before the first probe is thought to reflect the effects of repellent or deterrent surface factors. (2) The number of probes shorter than 3 min (test probes) that occur before the first phloem phase (E1), and (3) the minimum duration of waveform C within a probe before a phloem phase (a single E1 or an E12 period) likely reflect the role of epidermis/mesophyll and mesophyll/vascular parenchyma factors, respectively. (4) The time to first E1 and (5) the time to first sustained E2 (sE2 – any period of E2 longer than 10 min, previously called 'committed phloem ingestion' by Tjallingii, 1995) reflect the ease of phloem access and acceptance, respectively. Parameters (4) and (5) were calculated as time from the first probe in the recording. (6) The potential E2 index (van Helden & Tjallingii, 1993) was calculated as the percentage of time spent in E2 by an aphid with any sE2, after reaching the first sE2. The potential E2 index reflects how persistent phloem feeding is (7) the average duration of E2 periods for aphids with E2 (total time spent in E2 divided by the number of E2 events per aphid), and (8) the number and percentage of aphids showing sE2 per accession, reflect phloem as well as general plant suitability.

#### Statistical analysis

The EPG parameters were analysed individually for each aphid; the means and standard errors of the mean (SEM) of total number of aphids per *Solanum* genotype were calculated. To calculate parameters 5 and 6, only those aphids with E2 events were taken into account. For aphid colony development and probing and feeding behaviour the Mann–Whitney rank sum test was used to test for significance of the difference with the susceptible control *S. tuberosum* RH89-039-16 (SPSS 12.0.1 for Windows). Fisher's exact test was applied to analyse the number of aphids that had shown sE2 (software by Preacher & Briggs, 2001).

## Results

### Aphid colony-development test

Twenty *Solanum* genotypes were evaluated for susceptibility/resistance to *M. persicae*. Aphids were confined to one plant but were free to move around and choose any part of the plant for probing and feeding. Results of the aphid colony-development test on young plants were used to rank the plant species from susceptible to resistant (Table 1).

*Young plants.* When adult aphids are transferred from one host to a second one of a different species, it is expected that they will not be adjusted to that new host. In contrast, the nymphs produced by the adults will adjust. This is in agreement with our observations. On some plants of *S. tuberosum* RH89-039-16, *Solanum hondelmannii*, *Solanum jamesii* 18349-10, cultivar Kardal, *Solanum bulbocastanum*, and *Solanum spegazzinii*, none of the initial five adult aphids survived until the first scoring day (day 8). In contrast to this, newborn nymphs appeared to survive and successfully developed into adults on day 15. Genotypes *Solanum capsicibaccatum* 18268-2, *S. capsicibaccatum* 18268-5, *Solanum multiinterruptum*, *Solanum cardiophyllum*, *S. berthaultii* 20650-3, *Solanum stoloniferum*, *Solanum polyadenium*, and *S. tarijense* were highly resistant to *M. persicae*. On these genotypes fewer than five aphids (nymphs or adults) were found after 8 and 15 days, which is significantly fewer than on the susceptible control *S. tuberosum* RH89-039-16 (Mann–Whitney: $P<0.05$). A less striking reduction in colony development (moderate resistance) was observed for the genotypes *S. jamesii* 18349-1, cultivar Mondial and Kardal, and *S. spegazzinii.* On these genotypes only the number of nymphs on day 8 differed significantly from control *S. tuberosum* RH89-039-16 (Mann–Whitney: $P<0.05$) (Table 1).

*Flowering plants.* All plants showing susceptibility at the young stage of development were also susceptible to aphids at the mature stage. However, some of the plants that were resistant at the young developmental stage became susceptible at the mature flowering stage (Table 1). Some others remained resistant, even in the senescent stage.

### Aphid probing behaviour on 20 *Solanum* genotypes

The EPG data were processed into about 25 parameters. Most parameters related to stylet pathway showed no differences between accessions and the control line. The most relevant parameters with differences were divided into four groups to infer tissue location of resistance factors against aphids; these include parameters indicative of (i) surface resistance, (ii) epidermis/mesophyll resistance, (iii) mesophyll/phloem resistance, and (iv) phloem resistance (see Table 2).

*Surface resistance.* Eleven genotypes showed a significantly postponed first probe with respect to the susceptible control. This time to the first probe parameter can be considered as indicative of resistance factors located at the plant's surface level (Table 2). Eight out of 11 genotypes also showed some level of resistance in the aphid colony-development test. Three of them, *S. berthaultii* 20650-3, *S. polyadenium*, and *S. tarijense*, carry glandular trichomes. The others, cultivar Kardal, *S. spegazzinii*, *S. capsicibaccatum* 18268-2, *S. capsicibaccatum* 18268-5, and *S. stoloniferum* do not possess glandular trichomes; therefore these genotypes must have a different mechanism of surface resistance.

*Epidermis/mesophyll resistance.* Probes shorter than 3 min can be considered as test probes during which stylets do not penetrate deeper than a few cell layers (penetration rate is about one cell layer/min). The minimum duration of waveform C, which is preceding a first phloem phase (E1, sieve element salivation) during a probe, mainly reflects mesophyll interactions. Therefore, a large number of test probes and a long time until the first phloem phase activity are assumed to indicate resistance factors in peripheral layers of the plant tissue (i.e., epidermis and mesophyll). Only three genotypes (*Solanum okadae*, *S. berthaultii* 20650-3, and *S. stoloniferum*) have significantly larger numbers of short probes when compared to the susceptible control, and in almost all genotypes the pathway preceding the first phloem phase lasted longer (Table 2, epidermis/mesophyll column). It should be noted here that *S. stoloniferum* was the only genotype with a significantly larger number and longer duration of the waveform F when compared to the susceptible control *S. tuberosum* RH89-039-16 (frequency of waveform F per aphid $2.6 \pm 0.59$ vs. $0.8 \pm 0.31$, respectively, and duration of waveform F $63.7 \pm 13.58$ vs. $13.3 \pm 6.73$ min, respectively; Mann–Whitney: $P<0.05$). Waveform F is associated with derailed stylet mechanics or stylet penetration difficulties (Tjallingii, 1990).

*Mesophyll/phloem resistance.* A long time before the first sieve element salivation (E1) and before the first sustained phloem sap ingestion (sE2) in the recording (i.e., time since the first probe) can be considered as indicative for mesophyll/phloem resistance factors. Genotypes *S. okadae*, *S. hondelmannii*, and *S. multiinterruptum* show a relative long time to the first E1 but not to the first sE2; and the other way around holds for *S. jamesii* 18349-10, *S. jamesii* 18349-1, and cultivar Kardal, which all show relatively long

**Table 2** Electrical penetration graph (EPG) results and the tissue location of aphid-resistance factors as inferred from the parameters shown. Values are means ± SEM of EPG parameters described in the Materials and methods

| | | | Surface | Epidermis/mesophyll | | Mesophyll/phloem | | Phloem | | Aphids with sustained E2 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Solanum* species | Identity number or cultivar name[1] | n[2] | Time to first probe (min) | Probes <3 min before first E1 | Minimum C prior to E1 (min) | Time to first E1 in experiment (min) | Time to first sE2 in experiment (min) | Potential E2 index % | Average period of E2 (min) | n | % |
| *S. okadae* | 18108-3 | 15 | 1.7 ± 0.3 | 20 ± 5* | 9.8 ± 1.0** | 114 ± 24* | 219 ± 34 | 60 ± 10 | 56 ± 24 | 10 | 66.7 |
| *S. tuberosum* | cultivar Eersteling | 19 | 2.9 ± 0.8 | 6 ± 1 | 7.3 ± 0.6* | 45 ± 11 | 237 ± 31 | 55 ± 9 | 26 ± 7 | 10 | 52.6 |
| *S. phureja* | 17667-1 | 16 | 4.3 ± 1.0* | 7 ± 1 | 9.4 ± 0.9** | 60 ± 19 | 87 ± 18 | 61 ± 6 | 39 ± 6 | 16 | 100.0 |
| *S. tuberosum* | RH89-039-16 | 16 | 1.8 ± 0.3 | 7 ± 1 | 5.5 ± 0.6 | 58 ± 13 | 219 ± 36 | 73 ± 11 | 84 ± 33 | 10 | 62.5 |
| *S. hondelmannii* | 18182-2 | 15 | 11.6 ± 2.7** | 8 ± 1 | 8.1 ± 1.3 | 167 ± 26** | 260 ± 27 | 87 ± 9 | 80 ± 23 | 9 | 60.0 |
| *S. jamesii* | 18349-10 | 18 | 6.5 ± 3.0 | 6 ± 1 | 5.6 ± 0.6 | 51 ± 9 | 359 ± 1** | 100[3] | 4 ± 2* | 1[4] | 5.6 |
| *S. berthaultii* | 20644-6 | 16 | 4.5 ± 1.2 | 11 ± 3 | 8.9 ± 1.0** | 97 ± 24 | 181 ± 25 | 73 ± 6 | 48 ± 14 | 14 | 87.5 |
| *S. bulbocastanum* | 17693-2 | 16 | 8.3 ± 2.3* | 4 ± 1 | 7.4 ± 0.6* | 39 ± 9 | 287 ± 29 | 80 ± 9 | 56 ± 27 | 6 | 37.5 |
| *S. jamesii* | 18349-1 | 17 | 1.7 ± 0.4 | 9 ± 2 | 5.2 ± 0.3 | 62 ± 14 | 346 ± 8* | 88 ± 3[3] | 6 ± 2** | 2[4] | 11.8 |
| *S. tuberosum* | cultivar Mondial | 19 | 8.7 ± 4.5 | 4 ± 1 | 9.7 ± 0.8** | 49 ± 8 | 284 ± 20 | 75 ± 10 | 29 ± 9 | 11 | 57.9 |
| *S. tuberosum* | cultivar Kardal | 16 | 7.2 ± 2.8* | 10 ± 2 | 12.5 ± 1.4** | 81 ± 23 | 336 ± 19* | 99 ± 1[3] | 13 ± 10* | 2[4] | 12.5 |
| *S. spegazzinii* | 17839-2 | 15 | 6.5 ± 1.9* | 17 ± 5 | 6.9 ± 0.5* | 111 ± 29 | 285 ± 29 | 51 ± 15 | 31 ± 14 | 6 | 40.0 |
| *S. capsicibaccatum* | 18268-2 | 16 | 9.9 ± 3.0* | 17 ± 4 | 13.4 ± 2.8** | 194 ± 36** | 322 ± 21* | 27 ± 10 | 18 ± 5 | 3[4] | 18.8 |
| *S. capsicibaccatum* | 18268-5 | 15 | 15.7 ± 3.9** | 10 ± 3 | 12 ± 1.8** | 146 ± 33* | 349 ± 9* | 54 ± 46[3] | 6 ± 5* | 2[4] | 13.3 |
| *S. multiinterruptum* | 17829-2 | 19 | 5.0 ± 1.3 | 13 ± 3 | 7 ± 0.4** | 96 ± 13* | 246 ± 29 | 19 ± 4** | 10 ± 1* | 9 | 47.4 |
| *S. cardiophyllum* | 18326-1 | 16 | 2.1 ± 0.4 | 10 ± 3 | 7 ± 0.7 | 68 ± 23 | 177 ± 33 | 64 ± 8 | 51 ± 13 | 12 | 75.0 |
| *S. berthaultii* | 20650-3 | 16 | 13.5 ± 3.1* | 14 ± 2* | 8 ± 0.9* | 95 ± 16 | 305 ± 19 | 53 ± 12 | 29 ± 11 | 7 | 43.8 |
| *S. stoloniferum* | 17605-4 | 18 | 8.6 ± 1.6** | 14 ± 2* | 10 ± 1.2** | 179 ± 27** | 311 ± 24* | 65 ± 19 | 39 ± 25 | 4[4] | 22.2 |
| *S. polyadenium* | 17749-1 | 15 | 20.4 ± 6.0** | 12 ± 3 | 8 ± 1.3* | 188 ± 40* | 358 ± 2** | 100[3] | 6 ± 3* | 1[4] | 6.7 |
| *S. tarijense* | 17861-8 | 16 | 23.9 ± 14.9* | 10 ± 2 | 12 ± 1.5** | 185 ± 34** | 360 ± 0** | 0[3] | 0* | 0[4] | 0.0 |

Dotted lines group species according to relative level of resistance with aphid colony development test (Table 1)

[1]Identity numbers from CGN followed by the genotype in vitro collection number (e.g., -2) or cultivar name (cultivar); RH89-03916, breeding line chosen as susceptible control (diploid line used in interspecific crossings with wild *Solanum*).

[2]n, EPG replicates; *P<0.05, **P<0.005, Mann–Whitney.

[3]The number of aphids (replicas) is lower than 2, thus statistical analysis is not possible (potential E2 index is the percentage of time spent on E2 after subtraction of the time needed to reach the first sE2 and is calculated only for aphids showing sustained E2).

[4]Fisher's test at the 6th hour of EPG recording: P<0.05.

times to the first sE2 but not to the first E1 (Table 2; mesophyll/phloem column).

*Phloem resistance.* The potential E2 index (percentage of time spent in E2 after the first sustained E2) and the average duration of E2 periods both reflect how persistent phloem feeding is; low values for these parameters are related to phloem-located factors of resistance. Seven genotypes (*S. jamesii* 18349-10, *S. jamesii* 18349-1, cultivar Kardal, *S. capsicibaccatum* 18268-5, *S. multiinterruptum*, *S. polyadenium*, and *S. tarijense*) showed a reduced average duration of E2 periods compared to the susceptible control but *S. multiinterruptum* is the only one with a potential E2 index lower than the susceptible control *S. tuberosum* RH89-039-16 ($19 \pm 4$ vs. $73 \pm 11$ min, respectively; Mann–Whitney: $P<0.05$; Table 2).

*Resistance without behavioural effects.* The EPG parameters did not indicate any resistance factors in genotype *S. cardiophyllum*, but nevertheless, in the colony-development test this genotype was a very poor host, especially as young plants. During the 6 h of EPG monitoring, 75% of the aphids reached sustained phloem ingestion (sE2) with an average of $51 \pm 13$ min spent on E2 (this was 62.5% and $84 \pm 33$ min on the susceptible control) (Table 2).

#### Aphid probing behaviour on *Solanum tuberosum* cultivars

The probing behaviour of *M. persicae* on two of the cultivated potatoes in this study showed differences from the susceptible *S. tuberosum* RH89-039-16 control plants. Although probing and feeding on cultivar Eersteling was very similar to the control, cultivars Mondial and Kardal appeared to have some degree of resistance to *M. persicae* (see aphid colony-development test, day 8; Table 1). However, the level of this resistance in cultivars Mondial and Kardal strongly depended on the development stage of the plants (Table 1).

#### Probing on *Solanum berthaultii* and *Solanum tarijense* with and without intact glandular trichomes

Removing the products of the glandular trichomes from leaves of *S. tarijense* and *S. berthaultii* 20650-3 made plants more accessible to *M. persicae.* On these 'cleaned plants', many aphids reached the sustained E2 phase (Table 3).

## Discussion

#### Aphid performance on young vs. flowering plants

Host acceptance by the aphids was strongly dependent on the developmental stage of the plants or leaves. On the genotypes *S. cardiophyllum*, *S. multiinterruptum*, and cultivar Mondial host acceptance by the aphid increased dramatically from young plants to flowering stage (Table 1). In *S. polyadenium*, the resistance level changed from highly resistant in young to moderately resistant in mature plants. In *S. stoloniferum* and cultivar Kardal, young leaves always remained resistant and aphids were never found on apical leaves. In contrast, on susceptible potato cultivars the performance of *M. persicae* was superior on young plants when compared to mature plants, which was presumably caused by a better nutritionally amino acid composition in the phloem of young plants (Karley et al., 2002). Furthermore, aphids can compensate for a nutritionally deficient sap with the provision of supplementary amino acids from symbiotic bacteria (Douglas, 1998; Douglas et al., 2001). In wheat and barley, attack by the aphids *Schizaphis graminum* and *Diuraphis noxia* induces a change in the phloem amino acid composition that seems to be nutritionally better for the aphids' performance (Sandström et al., 2000). Voelckel et al. (2004) found that *Myzus nicotianae* can elicit differential transcriptional changes in source and sink leaves of *Nicotiana attenuata*; the expression of constitutive defence genes differed between source and sink leaves possibly correlated with feeding site preferences by aphids. All of these suggest that in response to aphid attack, a specific set of genes is induced in the host plant. In order to elucidate aphid-induced effects and their complex interplay with constitutive defences and nutritional factors, further studies at the biochemical, physical, and transcriptional levels will be necessary.

#### Aphid probing behaviour on 20 *Solanum* genotypes

Although in the colony-development tests, effects of aphid–plant interactions and induced phenotypical plant changes may have played a role, the EPG results most likely relate to constitutive properties of the exposed leaves. It is important to realize that wired aphids cannot leave an unfavourable plant. Consequently, they may presumably probe longer and more frequently than free aphids would do on the same plant. Differences between susceptible and resistant plants may thus have a tendency to be underestimated by EPG analysis (Tjallingii, 1988). This would depend on the duration of EPG recording. The longer the EPG recording is continued after the time needed to decide to leave a plant by a free aphid, the more its resistance will be underestimated.

*Surface resistance.* Surface resistance is the first line of defence against attack and is especially important to avoid virus infection. A long time to the first probe mainly reflects the effects of mechanical or olfactory stimuli present at the leaf surface, i.e., at the cuticle-epidermis level, such as the presence of trichomes, repellent volatiles,

**Table 3** Electrical penetration graph (EPG) results of *Myzus persicae* adults feeding on *Solanum berthaultii* 20650-3 and *Solanum tarijense* 17861-8 with intact glandular trichomes and devoid of glandular trichomes content during a 6-h recording period. Values are means ± SEM of EPG parameters described in the Materials and methods

| | | EPG results and inference of the location of aphid-resistance factor in plant tissue | | | | | | | Aphids with sustained E2 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | EPG | Surface | Epidermis/mesophyll | | Mesophyll/phloem | | Phloem | | | |
| Species and treatment | n | Time to first probe (min) | Probes <3 min before first E1 | Shortest C prior to E1 (min) | Time to first E1 in experiment (min) | Time to first sE2 in experiment (min) | Potential E2 index % | Average duration of E2 (min) | n | % |
| *S. tarijense* intact trichomes | 15 | 11.6 ± 4.0** | 5 ± 1 | 14.1 ± 2.2 | 109 ± 30 | 360 ± 0.0** | 0[2] | 4 ± 1 | 0[1] | 0.0 |
| *S. tarijense* no glandular trichomes | 15 | 1.6 ± 8 | 8 ± 3 | 10.6 ± 1.2 | 68 ± 15 | 311 ± 16 | 37 ± 11 | 26 ± 9 | 7 | 46.7 |
| *S. berthaultii* intact trichomes | 15 | 12.1 ± 4.0** | 5 ± 2 | 12.9 ± 2.1 | 85 ± 22 | 360 ± 0.0** | 0[2] | 3 ± 1** | 0[1] | 0.0 |
| *S. berthaultii* no glandular trich | 15 | 4.0 ± 1.7 | 7 ± 2 | 12.4 ± 1.5 | 82 ± 24 | 217 ± 30 | 40 ± 11 | 68 ± 23 | 10 | 66.7 |

*P<0.05, **P<0.005, Mann–Whitney.

[1]P<0.05 Fisher's test at the 6th hour of EPG recording.

[2]The number of aphids showing sustained E2 (replicas) is 0, thus statistical analysis is not possible (potential E2 index is the percentage of time spent on E2 after subtraction of the time needed to reach the first sE2 and is calculated only for aphids showing sustained E2).

colour, or toughness of the leaf surface (van Helden & Tjallingii, 1993). Clear surface resistance was observed in the glandular trichome containing *S. berthaultii* 20650-3, *S. polyadenium*, and *S. tarijense.* The role of glandular trichomes in the defence against insects is well documented (Gibson, 1971, 1974, 1976; Gibson & Turner, 1977; Tingley & Laubengayer, 1981). It is interesting to notice that the *S. berthaultii* 20650-3 genotype has a high resistance to *M. persicae*, whereas *S. berthaultii* 20644-6 appeared to be as susceptible as the control *S. tuberosum* RH89-039-16 (Tables 1 and 2). Variation in resistance level from totally susceptible to highly resistant within the same plant species has been described for different *Solanum* genotypes (Radcliffe & Lauer, 1968). Traits may segregate within accessions; thus, different seedlings could have significantly different genetic properties (Bamberg et al., 1994). Aphids on *S. berthaultii* 20644-6 did not show any delayed first probe, suggesting that at least part of its susceptibility might be explained by the lack of surface components, which are so typical for *S. berthaultii* in general. The other species, e.g., *S. spegazzinii*, *S. capsicibaccatum* 18268-2, *S. capsicibaccatum* 18268-5, and *S. stoloniferum*, as well as the cultivar Kardal, do not possess glandular trichomes; therefore, these genotypes must have a different mechanism of surface resistance. The basis of the observed surface resistance remains to be elucidated.

*Epidermis/mesophyll resistance.* Although only *S. berthaultii* 20650-3 and *S. stoloniferum* showed an increased number of test probes before the first phloem activity, almost all genotypes analysed showed a prolonged pathway when compared to control plants (Table 2; epidermis/mesophyll column). In order to elucidate the cause of this prolonged pathway, further studies will be necessary. The number and total duration of the F waveforms were notably larger only in *S. stoloniferum.* Waveform F has been shown to occur in the mesophyll only (Tjallingii, 1987). Moreover, 73% of F waveform periods started during probes within 3 min, suggesting mechanical causes of these derailed stylet mechanics located in the first tissue layers (stylets penetrate about one cell layer/min).

*Mesophyll/phloem resistance.* Sieve element salivation (waveform E1) always precedes phloem sap ingestion (E2) and sustained phloem sap ingestion (sE2). Therefore, the first E1 can be considered as the first established sieve element activity. However, a sieve element may have been 'reached' and punctured earlier already. Nearly every cell encountered along the intercellular pathway to the phloem is punctured and sampled (reflected as potential drops in EPGs; Prado & Tjallingii, 1994). The cells in the vascular bundle are punctured more frequently than those in the mesophyll (Tjallingii & Hogen Esch, 1993) and salivation by the aphid (E1 waveform) presumably indicates sieve element 'recognition' and not merely contact with the sieve element. The first sustained sieve element ingestion (sE2) has been considered as committed phloem ingestion or 'sieve element acceptance' (Tjallingii & Mayoral, 1992). Both events are central in the host plant selection process by the aphid. Genotypes showing a relatively long time to the first E1 but not to the first sE2 (i.e., *S. okadae*, *S. hondelmannii*, and *S. multiinterruptum*) most likely have resistance factors at the mesophyll level. However, the resistance factor(s) acting on the stylet's pathway to the phloem can be overcome by the aphid, as the result of the colony-development test was similar to the susceptible controls (Table 1). In contrast, a relatively long time to first sE2 but not to first E1 most likely indicates resistance factors at the phloem level (i.e., *S. jamesii* 18349-10, *S. jamesii* 18349-1, and cultivar Kardal). Nevertheless, some aphids are able to overcome these resistance barriers and can develop successfully on these plants (Table 1). The aphid colony-development tests show that aphids on *S. jamesii* 18349-1 and *S. jamesii* 18349-10 must be able to feed, but the 6-h period of EPG recording appeared to be too brief for the aphids to achieve sustained phloem feeding (sE2).

The potential E2 index (van Helden & Tjallingii, 1993) gives an indication of the aphid reaction after having the first successful phloem sap ingestion, irrespective of how long it takes for the aphid to reach sustained E2 for the first time (Tjallingii & Mayoral, 1992). In *S. multiinterruptum*, the reduced E2 index suggests the presence of a chemical factor in the phloem sap of young plants that stopped ingestion and reduced further phloem feeding.

*Resistance without behavioural effects.* Electrical penetration graph parameters do not indicate any resistance in genotype *S. cardiophyllum*, but nevertheless there was no colony development of aphids on young plants of this genotype. Therefore, in young plants the resistance factor appeared to work in a delayed way, suggesting an antibiotic effect or nutritional deficiency of the phloem sap affecting the development and survival of aphids rather than a behavioural interference (Table 2).

#### Probing on *Solanum berthaultii* and *Solanum tarijense* with and without intact glandular trichomes

Electrical penetration graph results from *S. berthaultii* 20650-3, *S. polyadenium*, and *S. tarijense* plants with intact glandular trichomes (Table 2) suggest that resistance to *M. persicae* differs between these genotypes. *Solanum berthaultii* 20650-3 showed resistance at surface and first tissue layers while *S. polyadenium* and *S. tarijense* showed

resistance factors at nearly all tissue levels: epidermal, mesophyll, and phloem (Table 2). Our results of plants with washed leaves indicate that the resistance in *S. berthaultii* 20650-3 and *S. tarijense* mainly depends on the trichomes (Table 3). Bonierbale et al. (1994) reported an incomplete correlation between trichome phenotype and aphid resistance in the progenies of crosses between *S. tuberosum* and *S. berthaultii* and one of their explanations was that different mechanisms of resistance – independent of trichomes – might be expressed in the hybrids. Previous work showed that removal of the trichomes from *S. berthaultii* leaflets resulted in increased adult feeding initiation, preference, and consumption of foliage by the Colorado potato beetle, *Leptinotarsa decemlineata* (Yencho & Tingey, 1994). Furthermore, the removal of type B droplets on trichomes of *S. berthaultii* resulted in a decreased time to the first probe by *M. persicae* as compared to intact plants; in contrast, when type B exudates of trichomes were transferred to *S. tuberosum* cultivar, first probes were delayed (Lapointe & Tingey, 1984).

Resistance based on glandular trichomes in wild tuber-bearing *Solanum* spp. has some drawbacks for breeding. The expression of resistance in *Solanum berthaulthii* appeared to be (i) due to the interaction of several chemical and physical characteristics of glandular trichomes, (ii) a quantitative trait (Mehlenbacher et al., 1983), and (iii) linked to undesirable agronomic characteristics such as late tuber development (Kalazich & Plaisted, 1991; Bonierbale et al., 1994). Additionally, it was found that resistance in field experiments is lower than under glasshouse conditions, probably because of the repeated influx of colonizing aphids in field trials that depletes the type-A trichomes, which are not renewed. In addition, precipitation and overhead irrigation may wash away trichome secretions in mature and senescent foliage (Tingey et al., 1982).

Comparison of EPG parameters from *S. tarijense* plants with and without glandular trichomes indicates that a strong surface resistance may bias EPG parameters associated with resistance at deeper tissue levels. Thus, the trichome effects completely prevented sustained phloem feeding, although some short periods of phloem ingestion may have occurred (Tables 2 and 3). It is likely that the glandular secretions and tarsal irritation interfered persistently with probing activities. The presence of a second line of defence depending on other mechanisms could not be demonstrated in *S. berthaultii* 20650-3 and *S. tarijense*.

#### Aphid colony development in relation to feeding behaviour

When interpreting EPG results, we should take into account that the differences observed between each test accession and the *S. tuberosum* control represents fine details of duration and occurrence of events or activities. An aphid can spend more or less time in activities on its stylet's path to the phloem, but the ultimate time of phloem feeding can be similar or longer. This final settling may occur after the 6 h we used in our EPG experiments. Therefore, the colony-development tests cover a completely different time frame than the initial events recorded by EPGs.

In studying plant resistance to aphids, EPGs of initial events of probing and feeding behaviour and colony-development tests of the overall plant suitability should be considered as complementary techniques, rather than parallel approaches. When we combined colony development results with EPG data on probing and feeding some paradoxical results appeared: EPGs from aphids feeding on *S. okadae*, *Solanum phureja*, cultivar Eersteling, *S. hondelmannii*, *S. jamesii* 18349-10, *S. berthaultii* 20644-6, and *Solanum bulbocastanum* showed that aphids encountered some constraints at different tissue levels when compared to the susceptible control, *S. tuberosum* RH89-03916. The aphid colony-development test showed that these accessions are similar in susceptibility to *S. tuberosum* RH89-03916. Thus, suitable host plants may contain resistant features at some tissue levels, but this resistance does not affect overall susceptibility.

Within the *S. tuberosum* varieties analysed, Mondial and Kardal showed some resistance with respect to colony-development at the young stage (Table 1) and EPG results showed factors at the surface, mesophyll, and phloem levels. On our susceptible control *S. tuberosum* RH89-039-16, some individual aphids on young fully expanded leaves also showed an EPG pattern indicative of resistance, similar to Kardal, although on average, the effect was low and aphid colonies developed well. Other studies have reported antibiotic resistance to *M. persicae* in some *S. tuberosum* varieties (Radcliffe & Lauer, 1968; Bintcliffe & Wratten, 1982). On the basis of our present knowledge however, we think that these 'antibiotic' effects might have been caused by avoidance of phloem feeding, i.e., 'antixenotic' effects at the phloem level. As pointed out by Karley et al. (2002) and in accordance with our results, many cultivated potato plants seem not to be optimal hosts for *M. persicae*. Most EPG data about resistance point in the direction of antixenotic effects, i.e., delays in showing phloem activities or no sustained phloem activity at all, which might be interpreted as phloem avoidance. The wired aphids could not leave the plants. Our aim was to study probing and feeding when aphids had access to the plants and how that would affect their plant penetration behaviour. Also free aphids have been observed to probe into non-host, resistant, or partially resistant plants frequently and even may spend considerable time on them. As long as they stay on such

plants, feeding will be reduced, leading to reduced performance, which has been the classical criterion for antibiosis. As we learn more about the details of host plant resistance to aphids, Painter's terminology (Painter, 1951) is often not very appropriate. However, one exception should be made here for *S. cardiophyllum*, on which aphid colony development was very poor despite the absence of evidence in the EPGs that probing and feeding were affected. Consequently, resistance in this genotype appears to be due to antibiosis sensu stricto.

#### Virus transmission

The partial plant resistance present in cultivar Kardal and *S. stoloniferum* can be important for limiting the transmission of phloem restricted viruses such as PLRV. Slow aphid population build-up on young plants will reduce secondary virus spread. Therefore, it would be interesting to elucidate the mechanism of the resistance in young leaves. Furthermore, the spread of viruses can be reduced by combining young plant resistance to aphids with 'mature plant resistance' (MPR) to viruses. Beemster (1987) showed that the older the plant at the time of inoculation, the fewer viruses are translocated to the tubers.

In general, vector efficiency of *M. persicae* will be reduced when the normal feeding behaviour is impaired. Genotypes with surface resistance factors combined with mesophyll- and phloem-localized resistance factor(s) can be expected to have a lower probability of inoculation by persistently transmitted viruses such as PLRV. Genotypes on which a small percentage of aphids reach sustained phloem ingestion and on which E2 has shorter duration (phloem factor resistance) are likely to lower the efficiency of aphids to acquire persistent virus.

## Conclusions

The combination of an assay for free aphid colony development with the EPG measurement of initial probing and feeding has been shown to be an effective way to obtain information on the location of resistance factors in tuber-bearing *Solanum* genotypes. Mechanisms of resistance to *M. persicae* were detected at the surface and at three plant tissue levels.

The expression of resistance to *M. persicae* varies with the age of the plants and with plant parts. Some of the plants that are resistant at a young developmental stage became susceptible at the flowering mature stage, either by a change in the whole plant or by reduced resistance in senescent leaves. We did not include other aphid species in this study. Therefore, we can only speak about resistance to *M. persicae*. Resistance to one aphid species often has no implication for resistance to other species. Van Helden & Tjallingii (1993) investigated a case of total resistance to *Nasonovia ribisnigri* in lettuce, which implied no resistance whatsoever to *M. persicae* or *Macrosiphum euphorbiae*.

The results of the present study were obtained under greenhouse and laboratory conditions, and can be used as a basis for further unravelling of resistance mechanisms at the metabolic, molecular, and genetic levels. Field experiments will be necessary to evaluate resistance under agricultural crop conditions.

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