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Characterization of *Solanum chomatophilum* resistance to 2 aphid potato pests, *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae* (Sulzer)

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ABSTRACT

The aphids *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) are responsible for yield reduction in potato (*Solanum tuberosum*) production by direct phloem feeding and by spreading viruses. Breeding resistant traits from *Solanum chomatophilum* into the potato germplasm provides alternative means to control aphid infestations. Integrated pest management strategy, using plant resistance, benefits from the characterization of the resistance and of its impact on aphid biology. Our objective was to characterize the resistance of *S. chomatophilum* by assessing the effects of accessions, plant parts on aphid performance, and by assessing the impact of the resistance factors on different aphid developmental stages and on alate morph production. Detailed aphid performance was obtained by measuring fecundity, survival, percentage of nymphs that reached adult moult, and population growth using whole plant and clip cage experimental designs. Accession and plant physiological age, but not aphid developmental stage, influenced all life-history parameters, except for alate morph production which was not induced on the resistant accessions. Plant part influence was independent of plant species and accession. Both experimental designs resulted in congruent resistance levels at the accession level for each of the two aphid species, supporting the use of any of them in *S. chomatophilum* resistance screening. PI243340 was resistant to both aphid species, while PI365324 and PI310990 were also resistant to *M. euphorbiae* and *M. persicae*, respectively.

1. Introduction

Macrosiphum euphorbiae (Thomas) and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) are major pests of the cultivated potato, *Solanum tuberosum* (Radcliffe, 1982; Blackman and Eastop, 1994). Both aphid species can be responsible for important yield loss by consuming phloem sap (Radcliffe, 1982) and through the spread of potato viruses (Radcliffe and Ragsdale, 2002). Aphid infestations are commonly controlled by insecticide applications but the rise of environmental concerns (Devine and Furlong, 2007) and the risk of evolution of insecticide resistance (Foster et al., 1998; Anstead et al., 2005) have led to the search for alternative strategies to control aphid populations. The breeding of resistant potato plants, which would negatively affect the performance of aphids, is one method that can achieve this goal (Smith and Quisenberry, 1994; Flanders et al., 1999). The cumulative effects of plant resistance and natural enemies can maintain aphid damages below an economic threshold (Dreyer and Campbell, 1987; Panda and Khush, 1995; Figueira and Fernando, 2004; Davis et al., 2007; Shannag and Obeidat, 2008).

Among the wide diversity of wild tuber-bearing *Solanum* species, some possess resistance to aphids and can hybridize with *S. tuberosum* (Spooner and Bamberg, 1994). *Solanum chomatophilum* has been identified as a genetic source of resistance to aphids for breeding programs (Radcliffe et al., 1981; Flanders et al., 1992; Flanders et al., 1997; Le Roux et al., 2007). *M. euphorbiae* and *M. persicae* display a lower fitness on *S. chomatophilum*, as estimated by aphid population counts and intrinsic rate of increase, compared to *S. tuberosum* (Radcliffe et al., 1981; Le Roux et al., 2007). However, variation in aphid resistance levels among different accessions of the same wild *Solanum* species has been observed (Radcliffe et al., 1981; Flanders et al., 1992), and thus resistance assessments should be conducted at the accession level. Furthermore, plant part influences aphid biological performance and thus should be considered when evaluating resistance level (Duncan and Couture, 1956; Guldemond et al., 1998; Le Roux et al., 2008). Determining the impact of plant resistance factors on aphid biology is important to predict the efficiency of the resistance factors in a pest management context. Resistance factors may have dissimilar effects on different insect

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developmental stages, as reported for Colorado potato beetle on wild *Solanum* species (Pelletier et al., 1999). Colonization of less suitable crop plants might induce the production of alate (winged) morphs in aphids (Gibson, 1971; Muller et al., 2001), which is not desirable because alate morphs can rapidly spread potato viruses (Blackman and Eastop, 1994).

Different methods have been used to assess aphid resistance. Some studies were conducted on excised leaves (Sams et al., 1975), others used clip cages to restrict aphids to certain parts of the plant in a controlled environment (Le Roux et al., 2004, 2007; Davis et al., 2007) and others were conducted in the field (Radcliffe et al., 1981; Flanders et al., 1992; Davis et al., 2007). Cutting a leaf triggers many physiological changes that can affect resistance (van Emden and Bashford, 1976). The clip cage method can impair leaf photosynthesis (Crafts-Brandner and Chu, 1999) and also results in a resistance value that is only valid for that part of the plant (Guldemond et al., 1998). However, the clip cage method has the advantage of enabling the measurement of parameters related to a single aphid, such as fecundity and survival, on a specific part of the plant, which may help to reveal the location of resistance factors. Field evaluations attempt to simulate agricultural conditions and include the effects of abiotic factors, natural enemies, and plant resistance. However, field trials are costly and time-consuming, making them unrealistic tools for high throughput screening. The resistance level may also be biased by variation of uncontrolled factors, as indicated by inconsistent results among different years (Davis et al., 2007). Timed sampling procedure, based on the number of aphids an observer counts in a given time (Radcliffe and Lauer, 1966), reduces the cost and the time associated with field studies, but neither assess the variation of resistance level within the plant nor the impacts of plant resistance on aphid biology. Finally, we favoured two methods in a controlled environment: clip cage and a protocol assessing the population growth on entire plants. This last method alleviates the drawbacks of clip cages by being less stressful to the plants and by assessing aphid preference within plants as well.

Our objective was to characterize the resistance levels of seven accessions of *S. chomatophilum* to *M. euphorbiae* and *M. persicae*. Survival and fecundity starting with 1st instar nymphs and alate adult aphids on differently aged leaves were measured with clip cages. Aphid population growth and the proportion of adult aphids that were alate were measured on entire plants while taking into account the impact of the physiological age of the plant part on which they were feeding. *S. tuberosum* (var. Shepody) served as a susceptible control in all experiments.

2. Materials and methods

2.1. Insects and plants

S. chomatophilum accessions (PI243340, PI243341, PI266387, PI310943, PI310990, PI365324 and PI365327), selected for their potential resistance against aphids (Radcliffe et al., 1981), were first grown from true seeds obtained from the USDA (US Potato Gene-Bank, Sturgeon Bay, WI, USA) and then two to three plants from each accession were propagated vegetatively by cuttings. *S. tuberosum* plants [var. Shepody, which possesses a mild resistance level to *M. euphorbiae* and *M. persicae* comparatively to other potato varieties (Davis et al., 2007)] were grown from tubers. Tuber seeds were Elite II quality (McCain Produce Inc., Florenceville, NB, Canada), meaning that less than 0.1% of tubers were infected by viruses. *M. persicae* and *M. euphorbiae* colonies were started from *virginoparous* aphids collected on the potato fields surrounding the Potato Research Centre (Fredericton, NB, Canada) during the summer of 2000. Aphids were subsequently reared on potted *S. tuberosum* (var. Shepody) plants placed in cages (wood frame: 1 m high, 50 cm deep and wide, all sides and ceiling screened), allowing alate aphids to engage in flight. Young alate aphids, used in the experiments, were standardized by removing all alate aphids from the ceiling and walls of the wooden cage, and collecting the alate aphids present on the ceiling of the same cage 14 h later. It was assumed that alate aphids fly from the plant less than 24 h after the final ecdysis and do not take off once settled on a suitable plant (Robert, 1988). Alate morph production in the wooden cage was induced by crowding (Muller et al., 2001). Collected alate aphids were, thus, approximately one-day old. One day-old nymphs were selected following daily observations of alate aphids individually maintained with a clip cage on *S. tuberosum* leaves. All aphid manipulations were realized with a soft-bristled paint brush. The conditions for growing plants, maintaining the aphid colonies, as well as for all the experiments were 16 h:8 h (light: dark), 24 °C: 20 °C (day: night) at 50% relative humidity.

2.2. Whole plant experiment

For each aphid species, a single large 6 to 8 week-old plant (minimum height of 35 cm, flowering with senescing leaves on the basal half of the main stem) of each *S. chomatophilum* accession or *S. tuberosum* was enclosed in a wood frame cage (as above). Ten young alate adult aphids (obtained as explained above) were released in the test cage by placing the 20 gram plastic vial (Fisher scientific, Ottawa, ON) containing them on the soil of the potted plant. Twelve days later, the plant was divided in 2 parts with respect to physiological age and each part was sampled separately. The top (i.e., young) part, which included all plant parts on the distal half of main and secondary stems (distal and basal halves were separated with respect to the length of the stems), contained part of the mature leaves and all the young leaves and reproductive buds. Secondary stems that were shorter than half the length of the main stem contained only young foliage and were assigned to the top part of the plant. The bottom (or basal) part of main and secondary stems was assigned to the bottom (i.e., old) part of the plant. The numbers of nymphs and of alate and apterous (wingless) adults were counted in each part of each plant. All accessions were studied simultaneously and the position of the different accessions was randomized within the growth chamber between trials. Four replicates of each plant accession were carried out, except for the accession PI266387 for *M. euphorbiae* and the accessions 266387 and 365327 for *M. persicae* which were replicated 3 times because the plants were not available for one replicate.

2.3. Clip cage experiment

Six large 6–8 week old plants of each *S. chomatophilum* accession (except PI365327, which was not available at the time of the experiment) and *S. tuberosum* were used. On each plant, 3 mature leaves (located on the penultimate or last level from the apex, partly senescent) and 3 young leaves (the second or third level) were studied. One young alate or one 1-day old nymph (obtained as explained above) was placed on the abaxial side of each leaf studied (18 replicates per plant accession × leaf age × aphid developmental stage combination) and covered with a clip cage. Survival and fecundity was recorded every 2 days for 14 days for alate aphids and for 20 days for nymphs. The percentage of nymphs that reached the adult moult and the average daily fecundity were calculated for the 3 aphids developing on similar-aged foliage within each plant. Daily fecundity was calculated for aphids still alive at the time of sampling. All plants of all accessions were studied at the same time and were positioned randomly within the growth chamber.