PLANT RESISTANCE

Rapid Method to Screen Resistance of Potato Plants Against *Myzus persicae* (Homoptera: Aphididae) in the Laboratory

VINCENT LE ROUX,1 JULIEN SAGUEZ,1, 2, 3 CHARLES VINCENT,3 AND PHILIPPE GIORDANENGO1


ABSTRACT With the objective to develop a potato, *Solanum tuberosum* L., resistance program against aphids, we propose a rapid screening method with *Myzus persicae* (Sulzer) in the laboratory. We aimed to optimize the duration of the whole procedure and to decrease the frequency of measurements. In a first experiment, intrinsic rate of natural increase (*r_m*) values were compared between adult aphids reared throughout their entire life and adults reared only during a period equivalent to their prereproductive period. No significant differences were observed. In a second experiment, four groups of aphids were distinguished according to the sampling frequency, i.e., those whose biological parameters were evaluated every single, second, third, and fourth day. Except for the fourth-day experiment, the *r_m* values estimated on aphids reared on the three potato lines were not significantly different whatever sampling frequency of single, second, or third day used to check aphids. Thus, screening efforts in laboratory can be largely optimized by evaluating adult aphids only during a period equivalent to their prereproductive period and assessing *M. persicae* populations every third day. Our method is reliable and adapted to screen a large number of potato plants against *M. persicae* because it allows an average 70% reduction in the time required for the whole experimental process.

KEY WORDS *Myzus persicae*, aphid, potato resistance, intrinsic rate of natural increase (*r_m*), screening method

THE APHID *Myzus persicae* (Sulzer) has a serious impact on a large number of crops, particularly on potato, one of the most important crops in the world. Direct phloem sap feeding may cause yield losses (Radcliffe 1982, Blackman and Eastop 1984). The most important damage consists of transmission of up to 100 different plant viruses (Blackman and Eastop 1984), among which potato virus Y (PVY) and potato leaf roll virus (PLRV) are the most important (Robert et al. 2000, Basky 2002). *M. persicae* populations are mostly managed with synthetic insecticides. Because aphid populations submitted to strong selective pressure become resistant to insecticides (Field et al. 1994, Devonshire et al. 1998, Martinez-Torres et al. 1999, Foster et al. 2000), alternatives and sustainable pest management systems have to be developed.

Cultivated potatoes, *Solanum tuberosum* L., have been genetically selected mostly for production characteristics, and consequently genes conferring insect resistance have to be found in wild species (Sanford et al. 1984, Jansky et al. 1999), so as to enrich the cultivated gene pool (Esposito et al. 2002).

Before characterizing plant resistance, the first step is to select, among many accessions of wild species of potatoes, the most and less resistant potato lines by a screening method in the field or in the laboratory. Radcliffe and Lauer (1970, 1971) and Radcliffe et al. (1974, 1988) have developed screening methods in the field. Plants are infested with aphids, and populations are regularly assessed by counting individuals, the rate of population development being an index of resistance.

In the laboratory, aphid performance can be determined by several methods, such as counting the number of embryos in aphid females (Adams and van Enden 1972, Dewar 1977), measuring aphid abundance in whole plants (Guldemond et al. 1998, Kift et al. 1999), and estimating of the mean relative growth rate (MRGR) (van Enden 1969, Adams and van Enden 1972, Wojciechowicz-Zytko and van Enden 1995) and intrinsic rate of natural increase of population (*r_m*) (Birch 1948). The *r_m* estimation method is largely used by several authors (Wyatt and White 1977, Caillaud et al. 1995, Gatehouse et al. 1996, Fragoyiannis et al. 1998, Holopainen and Kössi 1998, Cherqui et al. 2003). Nymph survival, prereproductive period, adult survival, and fecundity are assessed daily.

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and allow to calculate the $r_m$ values according to Birch (1948). However, this method is tedious and therefore unsuitable for large screening programs.

Here, we propose a rapid laboratory method to optimize screening of potato plants infested by *M. persicae*. Our study aimed to optimize the duration of the whole screening procedure and to decrease the frequency of measurements.

Materials and Methods

**Plant Material.** Internode explants of *Solanum tuberosum* L. (Désirée) were transformed with an *Agrobacterium tumefaciens* strain C58/pMP90 containing an insect (*Phaedon cochleariae* F.) (Coleoptera: Chrysomelidae) chitinase gene. The neomycin phosphotransferase (*nptII*) gene was used as selectable marker, under the control of the viral CaMV 35S promoter, as described by Saguez et al. (2004). After micropropagation, transgenic lines and a nontransformed one of the same cultivar obtained by callus regeneration were acclimatized in a greenhouse at 20 ± 1°C under a photoperiod of 16:8 (L:D) h and cultured for 40 d before their use in experiments.

**Insects.** *M. persicae* was reared on nontransformed *S. tuberosum* plants (‘Désirée’) before being used for feeding experiments. The environmental chamber was maintained at 20 ± 0.5°C under a photoperiod of 16:8 (L:D) h to induce parthenogenesis. The rearing was initiated from a single virginoparous female collected in early summer 1999 from a potato field near Loos-en-Gohelle, France (50°27′27″ N, 2°47′30″ E).

**Feeding Trials.** Two transgenic (CH1 and CH9) and a nontransformed potato lines were used. Five nymphs aged <24 h were transferred in a microcage clipped on the lower face of a potato leaf at the third or fourth level from the apex. At least 11 replicates were carried out for each potato line. On the day of emergence, females were isolated in a microcage.

Biological parameters were determined as described by Alla et al. (2003). Briefly, prereproductive period, i.e., the period of time from birth until onset of reproduction, adult survival and fecundity were assessed daily. For each feeding trial, the intrinsic rate of natural increase ($r_m$) was calculated as

$$ \Sigma e^{-r \alpha/l_x} l_x m_x = 1,$$

where $x$ is the age, $l_x$ the age-specific survival, and $m_x$ the age-specific fecundity (Birch 1948). The Jackknife method (Meyer et al. 1986) was used to evaluate the variance of the $r_m$ with the “Petit” program (J. S. Pierre, unpublished software).

In a first experiment, $r_m$ values were compared between adult aphids reared throughout their entire lives and adults reared only during a period equivalent to their prereproductive period. In a second experiment, biological parameters were determined only during a period equivalent to their prereproductive period. Biological parameters were evaluated every single, second, third, and fourth day, and four groups of data were accordingly considered.

**Statistical Analysis.** The effect of sampling period was analyzed using the nonparametric Wilcoxon rank-sum test to compare the demographic parameters between aphids reared on their entire lives and aphids whose adults were determined only during a period equivalent to their prereproductive period. A one-way analysis of variance (ANOVA) by using Statistica 5.5 software (StatSoft, Tulsa, OK) was performed to test the effect of sampling frequency on the estimation of demographic parameters of aphids bred on the three potato lines. After ANOVA, the Scheffé test was used at $P < 0.05$ to detect statistical differences among the means.

**Results**

**Experiment 1.** Sampling Period. For the nontransformed and the two transgenic (CH1 and CH9) potato lines, no significant differences were observed for the $r_m$ values when aphids were estimated over the adult life span compared with those estimated only during a period equivalent to their prereproductive period; $n = 61$, $Z = 3.05$, $P > 0.05$ for nontransformed line; $n = 62$, $Z = 6.84$, $P > 0.05$ for CH1 line; and $n = 74$, $Z = 7.39$, $P > 0.05$ for CH9 line (Fig. 1). More than 98% of the $r_m$ value was explained.

**Experiment 2.** Sampling Frequency. Except for the fourth-day group, prereproductive period estimated on aphids reared on the nontransformed, CH1, or CH9 potato plants were not significantly different whatever sampling frequency (Table 1). Daily fecundity of aphids fed with the three potato lines evaluated every single second, third, or fourth day did not show any statistical difference. Statistical difference detected by the Fisher’s test was then due to the potato lines, not to the sampling frequency.

As a consequence of a shortened postembryonic developmental time for *M. persicae* determined with a four-day sampling frequency, the $r_m$ value was sig-

![Fig. 1. Average $r_m$ values of *M. persicae* reared on three potato lines. ■ $r_m$ values of adults estimated over their entire lives were compared with □, $r_m$ values of adult estimated over a period equivalent to their prereproductive period. Vertical lines associated with each bar are the SD.](image)
Table 1. Average (±SD) *M. persicae* demographic parameters for a sampling frequency of 1, 2, 3, or 4 d

<table>
<thead>
<tr>
<th>Demographic parameters</th>
<th>1 d</th>
<th>2 d</th>
<th>3 d</th>
<th>4 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prereproductive period (d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontransformed (n = 61)</td>
<td>10.7 ± 1.2a</td>
<td>10.2 ± 1.4a</td>
<td>9.9 ± 1.4a</td>
<td>9.2 ± 1.9b</td>
</tr>
<tr>
<td>CH1 (n = 62)</td>
<td>10.5 ± 1.2a</td>
<td>9.9 ± 1.4a</td>
<td>9.6 ± 1.2a</td>
<td>8.8 ± 1.6b</td>
</tr>
<tr>
<td>CH9 (n = 74)</td>
<td>10.1 ± 0.9a</td>
<td>9.7 ± 1.0a</td>
<td>9.2 ± 0.8a</td>
<td>8.3 ± 1.0b</td>
</tr>
<tr>
<td>Daily fecundity (nymphs/female/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontransformed (n = 61)</td>
<td>1.1 ± 0.1a</td>
<td>1.1 ± 0.1a</td>
<td>1.1 ± 0.1a</td>
<td>1.1 ± 0.1a</td>
</tr>
<tr>
<td>CH1 (n = 62)</td>
<td>1.5 ± 0.2a</td>
<td>1.4 ± 0.2a</td>
<td>1.4 ± 0.2a</td>
<td>1.4 ± 0.2a</td>
</tr>
<tr>
<td>CH9 (n = 74)</td>
<td>1.6 ± 0.2a</td>
<td>1.6 ± 0.2a</td>
<td>1.5 ± 0.2a</td>
<td>1.5 ± 0.2a</td>
</tr>
<tr>
<td>rₘ (females/female/d)</td>
<td>0.17 ± 0.02a</td>
<td>0.17 ± 0.03a</td>
<td>0.18 ± 0.02a</td>
<td>0.19 ± 0.03b</td>
</tr>
<tr>
<td>Nontransformed (n = 61)</td>
<td>0.15 ± 0.01a</td>
<td>0.19 ± 0.02a</td>
<td>0.19 ± 0.01a</td>
<td>0.21 ± 0.02b</td>
</tr>
<tr>
<td>CH1 (n = 62)</td>
<td>0.19 ± 0.02a</td>
<td>0.20 ± 0.02a</td>
<td>0.21 ± 0.02a</td>
<td>0.22 ± 0.02b</td>
</tr>
<tr>
<td>CH9 (n = 74)</td>
<td>0.20 ± 0.02a</td>
<td>0.21 ± 0.02a</td>
<td>0.21 ± 0.02a</td>
<td>0.22 ± 0.02b</td>
</tr>
</tbody>
</table>

Means in the same row followed by the same letter indicate no significant difference according to the Scheffe test (df = 3; F ≥ 0.05).

Discussion

With the objective to optimize screening program of potato resistance efforts in laboratory, rₘ values can be reliably estimated by assessing adult *M. persicae* only during a period equivalent to their prereproductive period and with a sampling frequency of 3 d.

In our first experiment, >98% of the final rₘ value was explained when adults were assessed only during a period equivalent to their prereproductive period, instead of entire life. These results are in accordance with those of DeLoach (1974), which reported 95% of the final rₘ values were explained with *M. persicae* reared on cabbage.

In our second experiment prereproductive period, daily fecundity and rₘ values were statistically identical for a sampling frequency of single, second, or third day, regardless of the potato line used to rear the aphids. However, for a sampling frequency of 4 d, the estimate for the prereproductive period and the rₘ values was unreliable.

Thus, a frequency of aphid assessment every third day provided a reliable estimation of the rₘ value.

Other methods are used to estimate biological parameters in the laboratory. MRGR developed by van Emden (1969) allows measurement of *M. persicae* performance because a relationship between fecundity and adult weight (Kempton et al. 1980) and between rₘ and MRGR (Guldemond et al. 1998) has been established. This method is less subject to “maternal influence” than fecundity or rₘ and better differentiates between the cultivars tested than rₘ (Wojciechowicz-Zytko and van Emden 1995), but it does not take into account demographic parameters of aphids.

Measuring population increases on whole plants provides more realistic values of rₘ than the Birch’s method (Guldemond et al. 1998, Kift et al. 1999). However, the former method is more time-consuming than microcages experiments and therefore limits the number of plants that can be tested (Guldemond et al. 1998).

The rₘ estimation adapted from Birch (1948) is efficient and largely used by several authors (Wyatt and White 1977, Calbault et al. 1995, Gatehouse et al. 1996, Fragoyiannis et al. 1998, Holopainen and Kössi 1998, Cherqui et al. 2003). Prereproductive period, adult survival, and fecundity, are taken into account and can be compared as well as the rₘ value. Because in microcages, aphids are not allowed to move on different parts of the plant, the rₘ value could be misestimated. Evaluation of the rₘ on aphids isolated in clip-cages in the laboratory is generally intended to highlight potato resistance of antibiotic nature. However, plant repellency or deterrency, also referred as antixenosis, also could induce changes of the intrinsic rate of natural increase.

Screening efforts in laboratory can be largely optimized by working only during an adult period equivalent to their prereproductive period and assessing *M. persicae* populations every third day. This method is reliable and adapted to screen a large number of potato plants against *M. persicae*, but have to be complemented with a screening program in the field because these two methods provide additional information related to antixenosis and antibiosis resistance. The egg-laying strategy of *M. persicae* consists of producing offspring early in its adult life. It is likely that our method could be applied for other aphid species having similar egg-laying strategy [e.g., * Macrosiphum euphorbiae* (Thomas)]. Comparisons of the sampling period demonstrated a saving of ~40% of the duration of the whole process. In addition according to sampling frequency study three times as many plants can be assessed at the same time. Thus, up to 70% of the experimental duration can be saved with our method.

Acknowledgments

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