European Journal of Plant Pathology **103:** 521–536, 1997.

Early intracellular punctures by two aphid species on near-isogenic melon lines with and without the virus aphid transmission (*Vat*) resistance gene

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Accepted 4 April 1997

Key words: Aphis gossypii, Myzus persicae, Homoptera, Aphididae, muskmelon, CMV, stylet, Electrical Penetration Graphs, non persistent virus transmission, insect resistance gene

Abstract

The *Vat* resistance gene (in *Cucumis melo* L.) inhibits the transmission of non-persistent viruses by *Aphis gossypii* Glover, but does not affect transmission by *Myzus persicae* (Sulz.). To see whether this difference was behaviourally determined, we investigated the stylet penetration behaviour of these two aphid species by recording EPGs (Electrical Penetration Graphs) of 8 and 20 min on two sets of susceptible and resistant isogenic melon lines. During the 20 min EPG study, inoculation with CMV (Cucumber Mosaic Virus) was also investigated. For both sets of isogenic lines, the two aphid species were able to detect the presence of *Vat*. The mean duration of individual intracellular punctures on the resistant genotypes was significantly reduced for both *M. persicae* and *A. gossypii* (–10% and –8% respectively for duration of pattern 'pd' in the 20 min experiment); this reaction appeared faster for *M. persicae*, a species for which melon was not a suitable host-plant. Therefore, in contrast to *Vat*'s anti-transmission effect, this behavioural effect was not aphid species-specific. Also, the frequency of intracellular punctures on the resistant genotypes was significantly reduced in *A. gossypii* (but not in *M. persicae*): on average, this frequency dropped from $\approx$0.65 pd.min^{-1} on the susceptible genotypes to $\approx$0.5 pd.min^{-1} on the resistant ones. It was concluded that (intracellular?) chemical cues were detected very early by aphids probing on the resistant genotypes carrying the *Vat* gene. However, a comprehensive analysis of the behavioural traits of both aphids on the two genotypes could not alone account for the complete inhibition of transmission which was found only to occur for *A. gossypii* on resistant genotypes. None of the differences detected (between aphid species or between plant genotypes) could account for the *Vat* phenotype, although they may explain quantitative differences in transmission efficiencies between aphid species. It was thus concluded that *Vat* effect was primarily chemically mediated. Finally, some intracellular punctures bearing typical subphases have been identified in both aphid species and were designated as 'long potential drops' (pd-L). For *A. gossypii*, these were observed early after plant contact and their mean duration was twice that of standard intracellular punctures ($\approx$8.5 s vs $\approx$4.2 s). Although not necessary for CMV inoculation, the duration of such phases was positively correlated with a high transmission efficiency by *A. gossypii* on the susceptible genotype. The nature of this pattern and a putative mechanism of action of the *Vat* gene are discussed.

Abbreviations: CMV – Cucumber mosaic virus; EPG – Electrical penetration graph; NS – Not significant; pd – potential drop; R – Resistant; S – Susceptible; *Vat* – Virus aphid transmission (resistance gene).

Introduction

Aphis gossypii Glover, the cotton/melon aphid, causes direct and indirect damage to many crops, of which cotton and cucurbits are the most important (Khush and Brar, 1991; Leclant and Deguine, 1994). However, host-plant resistance to this species has been investigated almost exclusively on cucurbits (Mac Carter and

Habeck, 1973; Kennedy et al., 1978; Lecoq et al., 1979; Shinoda and Tanaka, 1989; Kishaba et al., 1992; Collins et al., 1994), and only part of this work has been focused on resistance to virus transmission (Lecoq et al., 1979, 1980; Kishaba et al., 1992) and its genetic determinism (Pitrat and Lecoq, 1980, 1982). Such a resistance to virus transmission has to be distinguished both from resistance to the vector (classical insect resistance) and from resistance to the virus itself. All three components result in an overall protection against viral diseases, although through completely different mechanisms, which are largely unknown for the vector associated resistances. In the melon species *Cucumis melo* L., resistance to *A. gossypii* was described in the early 1970s (Kishaba et al., 1971), and led to the release of commercial cultivars bearing the AR (Aphid Resistance) gene (McCreight et al., 1984). However, it was only a few years later that resistance to CMV transmission was identified in this species (Lecoq et al., 1979), and the monogenic dominant gene responsible for this trait was named 'virus aphid transmission resistance' (symbol *Vat*).

Very little is known of the mechanisms of resistance to virus transmission by aphids. The *Vat* resistance to the aphid itself seems to be mediated by extractable chemical factors, disturbing aphid feeding behaviour both at the phloem and pre-phloem levels (Chen et al., 1996a). However, the most common behavioural analyses carried out by the Electrical Penetration Graph (EPG) method (Tjallingii, 1978) to study plant resistance to aphids are focused on a time-range measured in hours (usually 2–16), and are not pertinent for many virus transmission studies. Although early papers were published in this area (Hodges and McLean, 1971; McLean, 1977; Simons et al., 1977; Holbrook, 1978), recent works have clearly demonstrated the value of electronic monitoring of aphid behaviour in virus transmission studies, either for circulative viruses (Scheller and Shukle, 1986; Shukle et al., 1987; Fereres et al., 1990; Prado and Tjallingii, 1994) or for non-persistent transmission (Powell, 1991, 1993; Powell et al., 1992, 1995; Perez et al., 1996).

A study of the feeding behaviour of *A. gossypii* on melons containing the AR gene has already been published (Kennedy et al., 1978), but it was not related to virus transmission and was not performed using the more recent and precise EPG technique. Therefore, and this was planned independently in two locations (Lyon, France and Madrid, Spain) with different biological material, EPG experiments were carried out on a time-scale (8 and 20 min) more appropriate for correlation with non-persistent virus transmission, where the *Vat*-sensitive vector species *A. gossypii* was compared to *Myzus persicae* (Sulz.), a vector species not affected in its transmission efficiency by the presence of the *Vat* gene (Lecoq et al., 1980). In both experiments, aphid probing was compared on two sets of near-isogenic lines differing by the presence or absence of the *Vat* gene. The questions underlying these studies were related to short term host-discrimination (are these aphids able to detect quickly the presence of *Vat* gene products in the resistant lines?) and to differential probing behaviour of the two species (is there a behavioural difference between *M. persicae* and *A. gossypii* that might be related to the differential ability to transmit non-persistent viruses to *Vat*-genotypes?).

Materials and methods

Insects

In EPG Experiment 1, the aphids used were i) an *Aphis gossypii* clone labelled Ag-LM02-Cm, obtained from clone NM1 (G. Labonne, INRA Montpellier) collected on melon (*C. melo*) and described as clone 13 by Lupoli et al. (1992), and ii) a *Myzus persicae* clone Mp-LC02-Vf, which was derived from a parthenogenetic rearing on broad bean *Vicia faba* L. (Y. Bouchery, INRA Colmar, France). *A. gossypii* was reared on susceptible melon plants cv. 'Vedrantais' (21 °C; L:D 16:8). As melon is not a suitable continuous host for *M. persicae*, this aphid was reared on *V. faba* seedlings, cv. 'Aguadulce', in the same parthenogenetic-inducing conditions.

The aphids used for Experiment 2 (EPG-virus transmission assay) were from a clone of *A. gossypii* derived from a single virginoparous aptera collected on melon at 'El Ejido' (Almeria, Spain), and a clone of *M. persicae* also derived from a single virginoparous aptera collected at 'El Encin' (Alcalá de Henares, Madrid, Spain). *A. gossypii* was reared on melon plants cv. 'Regal', whereas *M. persicae* was reared on pepper plants (*Capsicum annuum* L.) cv. 'Yolo Wonder'. Both clones were reared at 22 °C (day) and 16 °C (night), under a 16:8 (L:D) photoperiod.

Plants

In Experiment 1, plants used were *C. melo* cv. 'Vedrantais' (susceptible line) or resistant cv. 'Margot' (from

source PI 161375). These lines were near-isogenic (9 backcross generations on the susceptible parent), and seeds were kindly provided by Dr Michel Pitrat from INRA Avignon. For aphid rearing and EPG experiments, plants were cultivated in soil pots at 21 °C and 80% r.h. under 16:8 (L:D) photoperiod in growth chambers, and used at the 4-leaf growth stage.

In Experiment 2, the melon plants were also two highly isogenic susceptible and resistant melon lines, that have been obtained within the Cucurbitaceae breeding program of Sluis & Groot Sandoz Seeds at 'El Ejido' Research Station (Almeria, Spain). The susceptible parent was of the 'Galia' type, and the resistant source was the same PI 161375 line. The seeds were kindly supplied by J.I. Alvarez. Plants were cultivated in soil pots in an environmental growth chamber at 14:10 (L:D) photoperiod, at a temperature of 26 °C (day) and 20 °C (night), and were used at the 2–3 leaf growth stage.

It is therefore probable that the two resistant lines used carry the same *Vat* gene, which confers them both resistance to the aphid vector and to virus transmission.

Virus

The CMV isolate used in Experiment 2 was the Val-CMV-24 described by Garcia Luque et al. (1984), and kindly supplied by Prof. J.R. Diaz-Ruiz (CIB-CSIC, Madrid, Spain). This CMV isolate was obtained from melon plants collected at Valencia (Spain). The virus was kept as dried leaf tissue at 4 °C.

CMV source plants

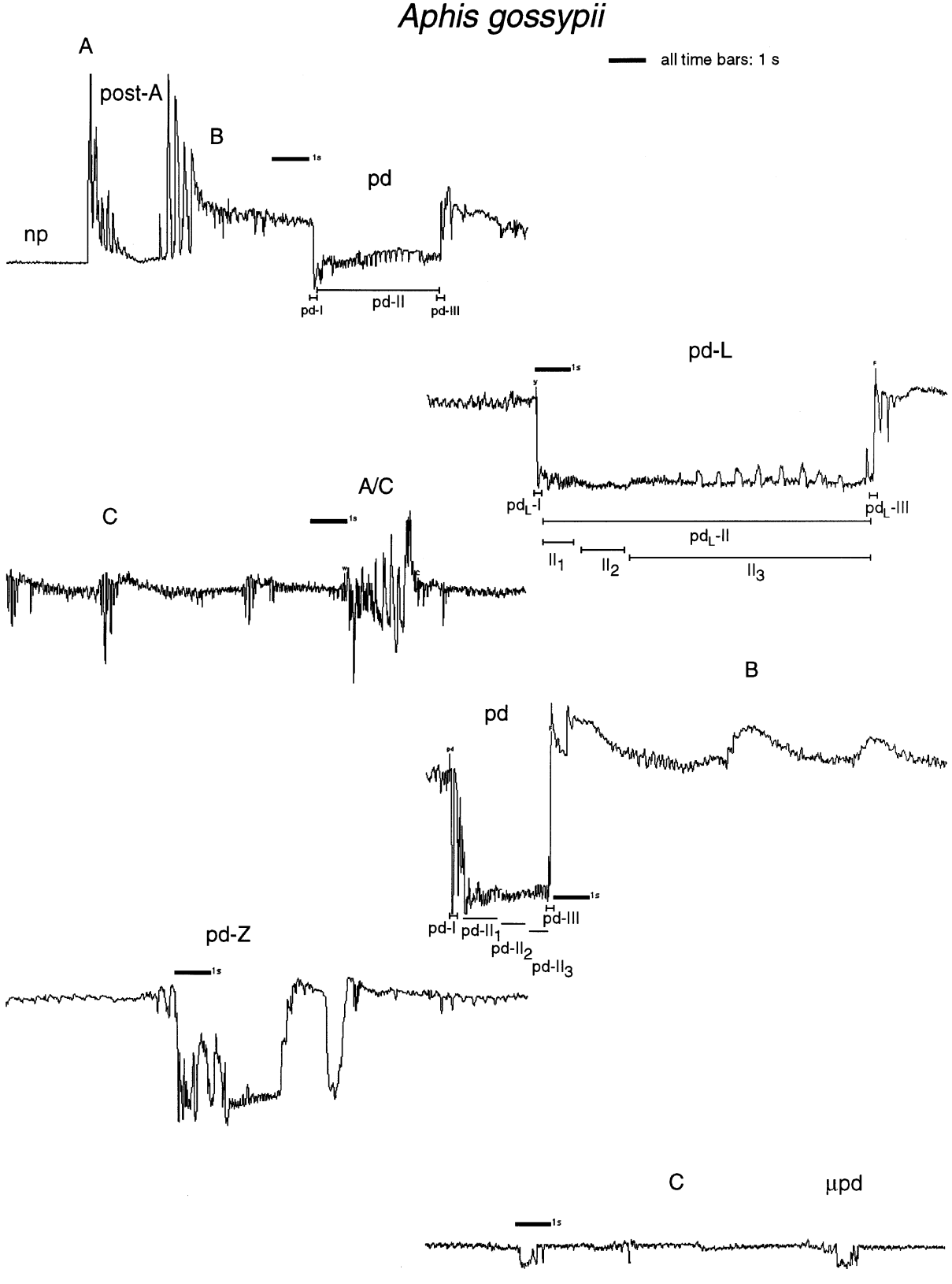
A virus infected source plant was used for acquisition of CMV in Experiment 2. Susceptible melons, cv. 'Canary yellow' were mechanically inoculated with the virus at the cotyledon stage. The plants developing the characteristic mosaic symptoms were used as virus source plants three weeks after inoculation.

EPG and virus transmission

The two experiments described were performed independently in two laboratories on different biological material (aphid clones and plant lines), and only then analysed in parallel. This explains the slightly different set-ups and methodologies of analysis. EPG amplifiers used were from Wageningen Agric. Univ. (Entomology Dept.). For both experiments, EPG recordings were acquired at 100 Hz through a MacAdios A/C-converter card and a MacIntosh acquisition software (G. Febvay, unpubl.); voltage adjustment was set to positive values during the first seconds of recording (all patterns shown were recorded using this standard set-up).

Experiment 1 (EPG). A. gossypii and *M. persicae* larvae were reared at low density to adulthood on susceptible host. The day before the experiment, young adults were wired with 3 cm long $\times$ 18 μm (ø) gold wire and kept on the host plant overnight. Before the EPG experiment, aphids were starved for one hour in Petri dishes with moist filter paper, and then put on test plants by groups of 4 (from one species, 2 on susceptible and 2 on resistant melon plants). Aphids were first correctly positioned on plants, and then gently pulled up and down by their wires to synchronise their access to plant (critical for the planned 8 min recording). Because of high individual variability, actual time before probing lasted 0 to 5 min, and such a parameter was judged quite artifactual, and was not taken into account in analyses (Van Helden and Tjallingii, 1992). Therefore, after recordings (10–20 min), an 8-min slice was cut from each EPG trace, beginning 1 s before first pattern A (electrical contact with plant tissues). Those few aphids which did not probe in 10 min were discarded. After completion of a recording series, aphids were discarded and replaced by individuals of the other species, on the same plant (and plants were changed after two series). Recording sessions were run daily from 10 am to 4 pm, and at least 28 valid repeats were finally analysed for each treatment.

Experiment 2 (EPG-virus transmission). Aphids were also reared at low density on susceptible plants. Young adult aphids were collected and wired to a 3 cm long $\times$ 20 μm (ø) gold wire. Then, aphids were kept in small plastic boxes and starved for one hour. Aphids were placed on the youngest fully expanded leaf of the CMV source plant and were subjected to a virus acquisition access period of 5 min. Afterwards, the wiring was completed by attaching the wired aphid to a copper wire 3 cm long. Finally, the viruliferous aphids were connected to the monitor probes and placed on the test plants ready to start the EPG recording. EPGs were recorded immediately after placing aphids on the test plants. Once the recording was completed, the plants and aphids were replaced by the following set. Only one aphid was allowed to probe on each test plant to determine the occurrence of CMV transmission by the aphid. Series of four sets (two aphid species-two melon lines) were monitored sequentially. Test plants


Aphis gossypii
all time bars: 1 s
A
post-A
B
pd
np
pd-I
pd-II
pd-III
pd-L
pdL-I
pdL-III
pdL-II
II1
II2
II3
A/C
C
B
pd
pd-I
pd-II1
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pd-II3
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C
µpd

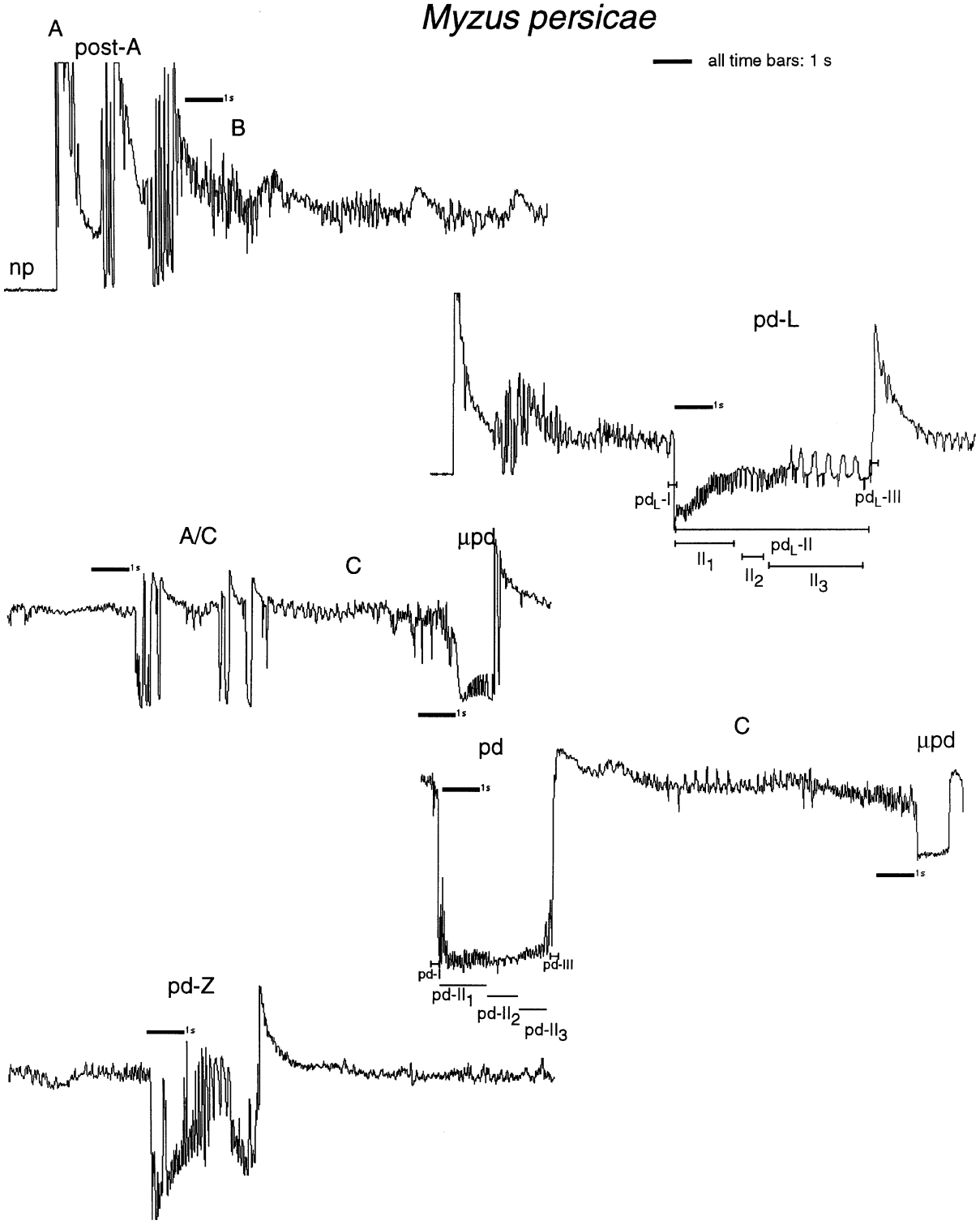

Figure 1. Examples of identified EPG patterns for the two aphid species used in this study, *Aphis gossypii* (left) and *Myzus persicae* (right). Time scale is shown and is similar for all patterns; Y scale (Volts) depends on amplifier gain and circuit properties during each recording, and is not strictly comparable in all figures. pd subphase nomenclature is that previously used by Powell et al. (1995), and has been retained for pd-L by homology.

were fumigated (Imidacloprid®, Bayer), transferred to aphid-proof cages in a greenhouse and checked for CMV symptoms after 2–3 weeks. Previous experiments showed that symptomatic plants of the melon lines used for these experiments were always infected with CMV, as assessed by ELISA (Fereres et al., 1995). EPGs were recorded for 20 min and all those aphids that did not start probing in 3 min were discarded. The non-probing time before a first probe was produced was excluded from data analysis to avoid high individual aphid variability. At least 25 recordings were analysed for each treatment.

For both experiments, analysis of EPG traces was achieved with the MacStylet software (Febvay et al., 1996), and calculation of standard or specific EPG variables on the identified patterns (Table 1; see results) was performed with commercial spreadsheet and the statistical softwares Statview 4 and SuperAnova 1.11 (Abacus Concepts, 1989). EPG variables for the different treatments were analysed using the Mann-Whitney U-test or an Anova, depending on data distribution. Patterns interrupted by the end of the experiment were included in all calculations, but were marked in the statistical files and the consistency of results (with and without these interrupted patterns) was checked. Only pattern C was affected by this bias, and mainly in the shorter experiment; means and significance of differences varied between the two modes of calculation, but the final trends displayed in our tables were always conserved.

Results

EPG patterns

After data collection, all EPG traces were viewed for recognition of patterns potentially useful in our short term analysis. In addition to standard patterns np, A, B, C and pd (Tjallingii, 1988), we defined *a priori* 5 more 'patterns' or variants (scored for both aphids), as illustrated in Figure 1 and Table 1.

For Experiment 1, all statistics were calculated on all patterns, but only the relevant variables are presented in subsequent tables. B pattern was retained, as indicative of an identifiable salivary sheath secretion, but patterns A, post-A or A/C were not further used because they were both not statistically discriminating and of unknown biological significance. Concerning potential drop patterns, we defined two additional subclasses, that were clearly identifiable with both aphids: the first one was the 'long potential drop' pattern, labelled 'pd-L', which was recently analysed for its correlation with potyvirus transmission (Powell et al., 1995); the second was labelled 'pd-Z' (abnormal pd) and defined as potential drops somehow interrupted by other behavioural items or unidentified 'noise', as illustrated in Figure 1. Very often, long potential drops occur as the first intracellular puncture after probing of the aphid, especially with *A. gossypii* (Figure 2), and display a characteristic sub-structure (Figure 1), with a subphase II3 containing several archlet/pulse structures (2 Hz) in addition to the background waveform (12–14 Hz); we used this arbitrary criterion (3 pulses or more) to differentiate pd-L from pd. Figure 3 shows the distribution of pd durations in both aphid species; for *A. gossypii*, pd duration was clearly bimodal, and this was mainly determined by the length of phase II3 (correlations between lengths of pd and pd-II3 was 0.98 for *A. gossypii*, and 0.94 for *M. persicae*). However, whereas phase pd-II2 was also significantly lengthened in pd-L (vs pd) for both aphids (whatever the melon genotype; data not shown), for *M. persicae*, the first phase of potential drops (pd-II1) was significantly *shortened* in pd-L punctures (Anova, $p = 0.0001$). This may be taken as an indication of distinct structure for pd and pd-L, even in *M. persicae* where distribution of total durations was not clearly bimodal (Figure 3).

In contrast to pd-L which bears a biological identity, pd-Z was only useful statistically in filtering off undesirable 'noise' from standard pd patterns; the statistical analysis of pd-Z revealed no significant feature, and it was not considered further in our EPG tables. The last non-classical pattern scored in our experiment was a pattern labelled 'μpd' (Figure 1). Although consisting of a clear potential drop in both aphid species, there is no indication of homology to other pd patterns (i.e. representative of a cell puncture). It is never a frequent event (Table 3, Table 5), but may be defined as a short (usually < 1s) and small (less than one third of a standard pd; Figure 1) potential drop. Unlike true potential drops, it has no specific sub-structure other than a characteristic high frequency (15–16 Hz for *A. gossypii* and 13–16 Hz for *M. persicae*).

From a descriptive point of view, *A. gossypii* and *M. persicae* displayed very similar (and mostly homologous) patterns in their first contacts with the plant tissues. The only *qualitative* traits that could distinguish the two species are the regularity of pd-L structure (clear-cut and longer in *A. gossypii*), and the features of

Table 1. Definitions of patterns scored in EPG analyses, and of non classical early EPG variables used (see Figure 1 for pattern examples)

Label of pattern or variable	Definition[1] of pattern or variable
patterns	
A	high amplitude pattern [*establishment of electrical contact*]
post-A	transient return to low voltage after electrical contact
B	periodic waves [*secretion of gelling saliva*]
C	composite extracellular signal [*intercellular stylet pathway*]
pd	"standard" potential drop [*stereotyped intracellular puncture*]
pd-L	long potential drop [*intracell. punct. with specific sub-pattern structure*]
pd-Z	abnormal or modified potential drop (not pd, not pd-L)
μpd	short and small "potential drops" (see text for comments)
A/C	unidentified high amplitude segments in C (pathway) pattern
np	absence of electrical contact [*non penetration*]
variables	
d-pd1	duration of first pd (from all pds *ie* pd, pd-L, pd-Z)
d-pd-L1	duration of first long pd (pd-L1)
d-np1	duration of first non-penetration period (after start of recording/probing[2])
t-pd1	time of occurrence of first of any pd (from start of recording/probing[2])
t-pd-L1	time of occurrence of first long pd (from start of recording)
t-np1	time of occurrence of first non penetration period (from start of recording)
Δt-pd1/np1	delay between first intracellular puncture and next non-penetration phase

[1]Most patterns and behavioural interpretations [*between brackets*] as defined by Tjallingii (1988).
[2]recordings were processed to start at first probe.

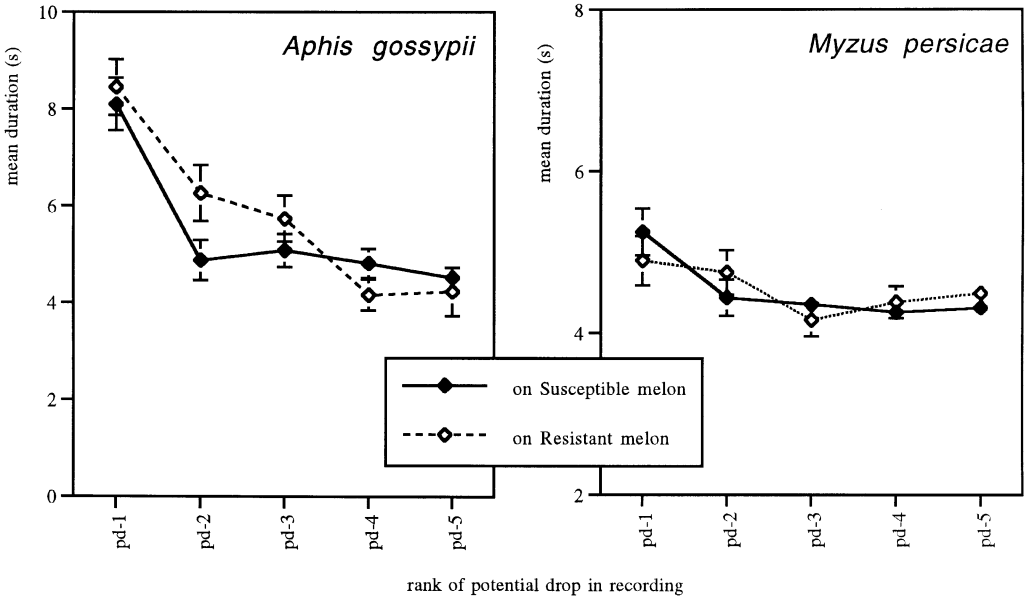

Figure 2. Evolution of duration of the first five intracellular punctures for both aphid species, according to their rank of occurrence in the probing sequence (pd & pd-L included; data from Experiment 1); plots are mean values and standard errors.

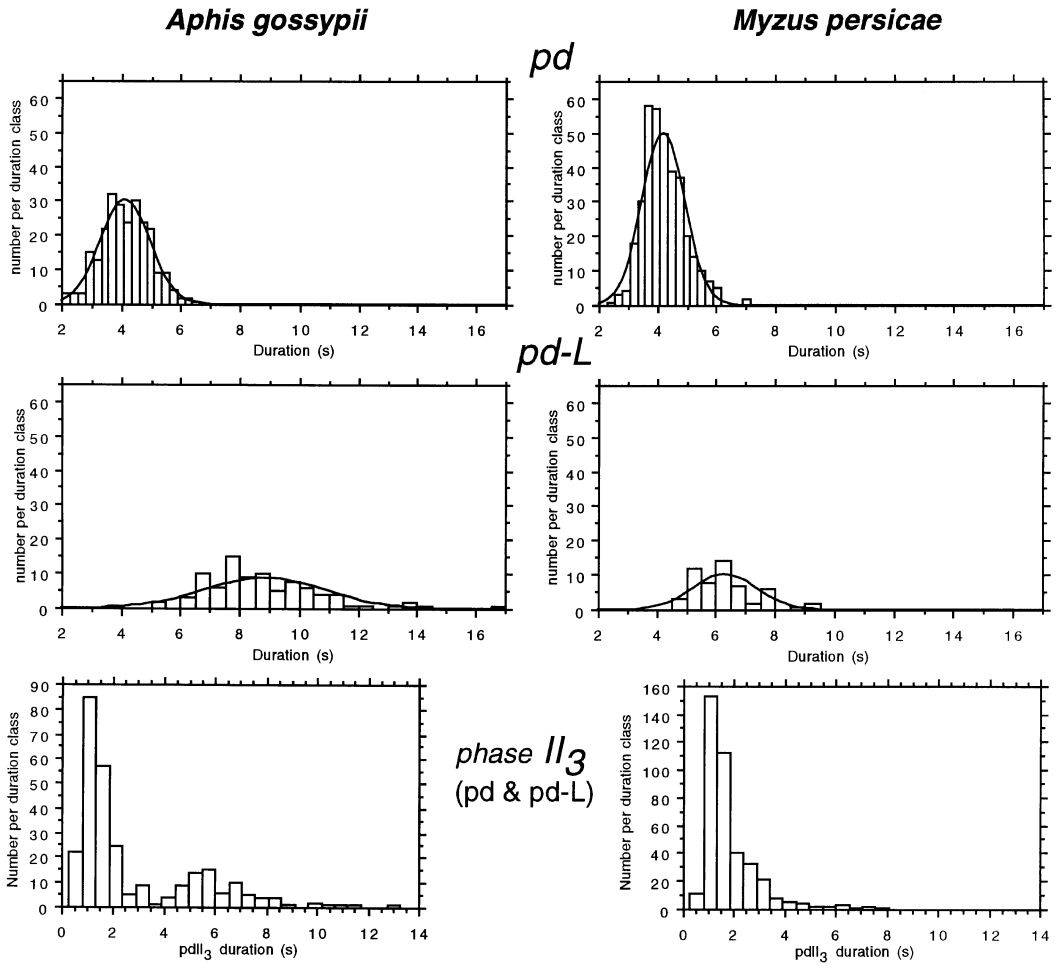

Figure 3. Distribution of potential drop duration for both aphid species (left/right) and potential drop type (upper row: pd / middle row: pd-L). The lower row plots the distribution of durations of subphase II3 for both pd types; data from Experiment 1.

μpd (clearly square-shaped in *M. persicae*; see Figure 1). No obvious biological correlation could be derived from these differences.

In Experiment 2, all the EPG traces were also closely reviewed in order to identify and quantify those patterns of potential biological significance, among the standard patterns and those described for Experiment 1. Since the time limits of certain patterns such as A or B were difficult to establish, we decided to include them here as part of waveform C, as commonly done in EPG analysis. Concerning potential drop patterns, we defined and computed pd-L, pd-Z and μpd using the same criteria as described for Experiment 1. The typical appearance of pd-L as the first intracellular puncture in the probe was again more frequently observed for *A. gossypii* than for *M. persicae*. In *M. persicae* traces, pd-L occasionally appeared as the second intracellular puncture after probing, or even at a later stage. This was also the case in Experiment 1 (compare t-pd1 and t-pd-L1, Table 3), and the difference between the two species appears clearly in the sequential plot of Figure 2.

Statistical analysis of individual pattern segments

For Experiment 1, Table 2 gives the mean durations and U-test analysis of the individual pattern segments. The duration of standard intracellular punctures (pd) appears to be shortened, slightly but significantly (–3.5%), for *M. persicae* on the resistant genotype, but not for *A. gossypii*. The trend was similar for the long potential drops pd-L (*A. gossypii*, NS; *M. persicae*, –

7%, p = 0.067). In addition, our analysis of sub-phase structure in Experiment 1 showed that the main contribution to the shortening of pd duration on resistant plants with *M. persicae* (Table 2) was due to phases II1 and II2 (Anovas, II1: S 1.75 s, R 1.66 s, p = 0.0076; II2: S 0.96 s, R 0.88 s, p = 0.0074), and not to phase II3 (S or R 1.4 s, p = 0.68; Anova NS). For other patterns, individual durations revealed significant differences between melon genotypes only for μpds of *A. gossypii* and for pathway (C) activities of *M. persicae* (Table 2): in this 8 min experiment, the probing sequences of *M. persicae* were much longer on susceptible than on resistant plants.

Table 4 shows the mean duration of the individual patterns scored in the EPG-virus transmission experiment. Here again, *M. persicae* displayed shorter intracellular punctures on the resistant melon genotype (pd, –9.8%; p = 0.0001). However, in contrast to Experiment 1, *A. gossypii* performed also significantly shorter pd on the resistant genotype (–7.8%; p = 0.0001). The different experimental durations may be responsible for the shorter mean pd duration for *A. gossypii* on the resistant genotype in Experiment 2. This is consistent with a correlation analysis done for Experiment 1 (duration of pd vs its time position in the 8-min acquisition sequence; data not shown), which showed a slightly positive slope for both species, as well as a higher slope for the susceptible genotype than for the resistant one. There seems to be a 'normal' lengthening of intracellular punctures, that is markedly impaired on the resistant genotype; this may indicate an adaptation of puncture to internal stimuli of host-plant. A similar reduction was observed again for the long intracellular punctures (pd-L, Table 4). Both aphid species exhibited a shorter pd-L when probing on the resistant genotype (–12.3%, p = 0.015 for *A. gossypii*; –3.8%, p = 0.12 for *M. persicae*). There was no evidence that aphids displayed any difference on susceptible vs resistant melon genotypes when the other behavioural variables were tested (μpd, np or C). We noted however that the C patterns lasted longer over time on the susceptible genotype. The last probe accomplished by *A. gossypii* in the 20 min recordings lasted more than 6 min in 66.7% of the EPG recordings on the susceptible genotype and only in 43.3% on the resistant genotype (resp. 39.3% and 35.7% for *M. persicae*). Thus, the *Vat* gene seems again to alter the 'normal' evolution of the aphid feeding process. Also, it should be pointed out that *M. persicae* succeeded in reaching the sieve elements and achieved phloem ingestion (waveforms E1 and E2 were observed) in 2 of the 25 EPGs recorded on the susceptible genotype. *A. gossypii* was unable to reach the phloem in the time of study (20 min).

Standard and sequential EPG variables

Table 3 reports statistical information on standard EPG variables for Experiment 1, including the frequencies of patterns in individual recordings, their total duration, and some additional variables related to early aphid/plant contact, as defined in Table 1. For *M. persicae*, the mean number of patterns C and np increased on the resistant genotype (i.e. probing was more interrupted on resistant plants), and occurrence of μpd differs between genotypes (although showing an increase for *A. gossypii* and a decrease for *M. persicae* on resistant plants). In addition, *M. persicae* showed more distinct salivation waveforms on the resistant genotype (3.3 vs 2.2 B phases per 8 min), a feature not displayed by *A. gossypii*. Conversely, the frequency of intracellular punctures decreased by 30% when *A. gossypii* probed on resistant melon (not for *M. persicae*). The second part of Table 3 concerns total duration of each pattern during the 8 min probe, and no clear trend could be observed with such variables (except for μpd with *M. persicae*, reflecting the difference already mentioned). However, one might observe the large difference in non-penetration time between *A. gossypii* and *M. persicae*: more than two interruptions (np) occurred for *A. gossypii*, representing more than 2 min outside plant tissues, whereas *M. persicae* frequently displayed one continuous penetration, especially on susceptible melons, and mean non-probing duration did not exceed one minute. Finally, the third part of Table 3 shows that none of the early parameters were able to discriminate melon genotypes significantly: for both aphid species, the first intracellular punctures (either pd or pd-L) did not differ in duration or in time of occurrence, as was the case of the first stylet withdrawal from plant tissues (d-np1 and t-np1), or for the time lag between the first intracellular puncture and the next end of probe (Δt-pd1/np1).

Table 5 reports the frequency and cumulative duration of the behavioural patterns associated to individual recordings for Experiment 2, as well as two of the early-occurring variables bearing potential virological interest (Table 1). Both aphid species exhibited an increased probing/non-probing frequency on the resistant genotype, i.e. higher frequencies of C and np patterns (not significant). This behaviour appeared to be associated with a longer probing time (C: *A. gossypii*. p = 0.024, *M. persicae* NS) and a shorter non-probing

Table 2. Experiment 1 – EPG variables related to mean duration (s) of *individual* events scored for the two aphid species *A. gossypii* and *M. persicae* on susceptible (Védrantais, S) and resistant (Margot, R) melon lines

	Aphis gossypii							*Myzus persicae*						
	S			**R**			test	**S**			**R**			test
Variables	Mean (s)	±*SE*	n	Mean (s)	±*SE*	n	p[1]	Mean (s)	±*SE*	n	Mean (s)	±*SE*	n	p
Gaussian variables														
pd	4.10	*0.07*	149	4.17	*0.09*	98	0.43	4.26	*0.05*	209	4.11	*0.06*	146	0.045
pd-L	8.57	*0.28*	47	8.19	*0.32*	42	0.16	6.55	*0.22*	24	6.10	*0.19*	31	0.067
	Median (s)	*range*[2]	n	Median (s)	*range*	n	p	Median (s)	*range*	n	Median (s)	*range*	n	p
non Gaussian variables														
μpd	0.73	*3.77*	11	1.27	*2.85*	18	0.027	1.08	*3.17*	55	0.98	*3.18*	15	0.37
np	44	*211*	60	38	*158*	57	0.80	41	*48*	8	29	*229*	25	0.22
B	5.7	*83*	152	6.9	*79*	147	0.32	14	*138*	68	11	*76*	90	0.18
C	32	*472*	100	52	*454*	85	0.38	417	*459*	39	184	*460*	53	0.0001

[1]Mann & Whitney U-test performed on all variables; probability of test underlined only if lower than 5% (equality rejected between R and S lines).
[2] range: *max* value – *min* value.

Table 3. Experiment 1 – general EPG variables related to individual recordings: number of patterns occurring in the 8-min period, total (cumulative) duration of patterns, and specific variables related to first contacts with plants

	Aphis gossypii							*Myzus persicae*						
	S			**R**			test	**S**			**R**			test
Variables	Mean	range[1]	n	Mean	range	n	p[2]	Mean	range	n	Mean	range	n	p
Number variables														
B	5.2	*12*	30	5.4	*10*	28	0.59	2.2	*5*	31	3.3	*10*	28	0.028
C	3.3	*6*	30	3.0	*5*	28	0.66	1.3	*1*	31	1.9	*3*	28	0.002
np	2.5	*7*	30	2.2	*5*	28	0.68	0.3	*1*	31	0.9	*4*	28	0.003
pd	5.0	*14*	30	3.5	*10*	28	0.050	6.5	*12*	31	5.3	*13*	28	0.16
pd-L	1.6	*4*	30	1.5	*4*	28	0.83	0.8	*3*	31	1.1	*6*	28	0.60
μpd	0.4	*6*	30	0.6	*5*	28	0.037	1.8	*7*	31	0.5	*2*	28	0.001
Total duration variables (s)														
B	62	*190*	30	73	*207*	28	0.093	46	*235*	31	50	*136*	27	0.10
C	241	*345*	28	254	*410*	27	0.74	372	*238*	30	345	*423*	28	0.26
np	159	*315*	25	135	*399*	23	0.22	40	*53*	9	63	*328*	18	0.92
pd	21	*56*	29	16	*39*	25	0.21	29	*52*	30	22	*56*	27	0.072
pd-L	16.1	*33*	25	17.6	*38*	22	0.58	9.3	*20*	17	11.1	*32*	17	0.96
μpd	2.8	*6*	4	2.2	*8*	11	0.95	3.0	*8*	22	1.7	*5*	11	0.017
Other (early) variables (s)[3]														
d-pd1	8.1	*10*	30	8.2	*12*	28	0.77	5.1	*6*	31	4.9	*5*	28	0.63
d-pd-L1	8.8	*8*	27	9.6	*8*	23	0.19	6.5	*4*	17	6.3	*2.8*	17	0.67
d-np1	73	*209*	25	53	*143*	23	0.11	44	*48*	8	51	*229*	17	0.42
t-pd1	18	*78*	30	42	*169*	28	0.16	33	*96*	31	46	*281*	28	0.52
t-pd-L1	18	*78*	27	36	*134*	23	0.16	65	*287*	17	51	*279*	17	0.13
t-np1	78	*433*	25	104	*373*	23	0.19	196	*445*	9	147	*398*	17	0.37
Δt-pd1/np1	60	*412*	25	66	*232*	23	0.17	165	*354*	9	125	*378*	17	0.37

[1] range: *max* value – *min* value.
[2] Statistical test performed for all variables: Mann & Whitney (U-test); probability of test underlined only if lower than 5% (equality rejected between R and S lines).
[3] As defined in Table 1.

Table 4. Experiment 2 – EPG variables related to mean duration (s) of *individual* events scored for the two aphid species on susceptible (Galia, S) and resistant (R) melon lines

	Aphis gossypii							*Myzus persicae*						
	S			**R**			test	**S**			**R**			test
Variables	Mean (s)	±*SE*	n	Mean (s)	±*SE*	n	p[1]	Mean (s)	±*SE*	n	Mean (s)	±*SE*	n	p
Gaussian variables														
pd	4.12	0.04	409	3.8	0.05	313	<0.0001	3.69	0.04	366	3.33	0.03	342	<0.0001
pd-L	8.83	0.45	25	7.74	0.40	25	0.015	6.29	0.15	35	6.05	0.17	34	0.12
	Median (s)	*range*[2]	n	Median (s)	*range*	n	p	Median (s)	*range*	n	Median (s)	*range*	n	p
non Gaussian variables														
μ**pd**	1.24	1.54	33	1.29	1.74	38	0.29	1.06	1.39	47	1.06	1.62	59	0.35
np	41	219	75	43	630	105	0.50	40	162	82	43	208	93	0.49
C	142	1195	103	99	1129	126	0.28	152	1194	99	124	1099	117	0.23

[1] Mann & Whitney U-test performed on all variables; probability of test underlined only if lower than 5% (equality rejected between R and S lines).
[2]range: *max* value – *min* value.

time (np, *A. gossypii*. p = 0.019; *M. persicae* NS) on the susceptible genotype. Here again, *A. gossypii* showed an increase in the frequency of standard cellular punctures (pd) when probing on the susceptible genotype (+22%; p = 0.045), as well as a longer mean total duration of these pd (+29.0%; p = 0.004). Although *M. persicae* exhibited a similar trend for these variables (pd frequency +5.2% and total pd duration +14.6% for the susceptible genotype), differences were not statistically significant. *A. gossypii* and *M. persicae* displayed similar frequencies and cumulative duration of long potential drops (pd-L) on both melon genotypes. Finally, the early-occurring variables were only marginally altered: on the resistant genotype, both aphid species were somehow delayed in displaying the first potential drop (t-pd1) and probing seemed to be interrupted earlier after the first intracellular puncture (Δt-pd1/ np1). For *A. gossypii*, the mean value of these two early-occurring variables was about twice as long on one genotype as on the other, but individual variability was very high and differences were not significant (Table 5).

Correlation with virus transmission

A. gossypii transmitted CMV to 12 of the 30 susceptible melon plants (40% transmission efficiency), while none of the resistant plants became infected. *M. persicae* was much less efficient, since only two of the 50 melon test plants became infected (one of each of the 25 resistant and 25 susceptible plants). This is in total agreement with previous data on the specificity of the *Vat* gene (Lecoq et al., 1979; Fereres et al., 1995).

The relationship between the transmission of CMV and the behavioural variables was analysed only for *A. gossypii* on the susceptible genotype. Table 6 shows the mean duration of the individual behavioural patterns on infected vs non-infected melon plants. An increase in the duration of long potential drops (pd-L; +29.5%) was observed for the plants that became infected. This trend is confirmed by a correlation analysis between pd-L duration and transmission efficiency (not shown): the longest intracellular punctures (pd-L $>$ 9 s) were very significantly associated with a high transmission efficiency (χ^2 = 13.2, p = 0.0003). However, CMV transmission was not always associated with pd-L pattern, and in 4 out of the 12 plants that became infected, no pd-L could be identified. No differences were noticed for other EPG patterns when infected and non-infected test plants were compared (Table 6).

The frequencies and the cumulative duration of the behavioural patterns associated with individual aphid records of *A. gossypii* probing on the susceptible genotype after virus acquisition were also computed and statistically analysed for comparison between infected vs non-infected plants (data not shown). No significant differences between treatments were found for any variable, although an increased activity of the aphids on plants that became infected has been noticed. Also,

Table 5. Experiment 2 – general EPG variables related to individual recordings: number of patterns occurring in the 20-min period, total (cumulative) duration of patterns, and specific variables of virological interest related to first contacts with plants

	Aphis gossypii							*Myzus persicae*						
	S			**R**			test	**S**			**R**			test
Variables	Mean	range[1]	n	Mean	range	n	p[2]	Mean	range	n	Mean	range	n	p
Number variables														
C	3.5	8	30	4.2	9	30	0.16	4.2	7	25	4.7	8	25	0.64
np	3.5	10	30	4.5	8	30	0.088	4	8	25	4.7	8	25	0.51
pd	13.4	19	30	10.5	18	30	0.045	14.5	26	25	13.7	19	25	0.89
pd-L	0.83	2	30	0.83	2	30	0.97	1.5	6	25	1.4	6	25	0.82
μpd	1.1	3	30	1.3	5	30	0.86	1.9	6	25	2.4	6	25	0.22
Total duration variables (s)														
C	1056	471	30	974	604	30	0.024	996	510	25	969	683	25	0.81
np	142	471	29	227	649	30	0.019	204	510	24	231	683	25	0.81
pd	56	86	30	39	76	30	0.004	53	97	25	46	66	25	0.29
pd-L	7.4	19	21	6.4	16	20	0.39	9.2	35	19	8.5	38	21	0.57
pd+pd-L	63	96	30	46	91	30	0.004	63	86	25	54	62	25	0.26
μpd	1.2	3.2	22	1.6	6.1	21	0.51	2.1	7.5	19	2.5	6.7	22	0.33
Other (early) variables (s)[3]														
t-pd1	13	81	30	24	248	30	0.37	9.7	44	25	12.3	64	25	0.93
Δt-pd1/np1	460	1099	30	197	1108	30	0.25	232	1177	25	166	558	25	0.92

[1]range: *max* value – *min* value.
[2] Statistical test performed for all variables: Mann & Whitney (U-test); probability of test underlined only if lower than 5% (equality rejected between R and S lines).
[3] As defined in Table 1.

Table 6. Experiment 2 – variables related to mean duration (s) of *individual* patterns of *A. gossypii* on infected and non infected susceptible test plants

	Aphis gossypii						
	Infected			Non infected			test
Variables	Mean (s)	$\pm$ *SE*	n	Mean (s)	$\pm$ *SE*	n	p[1]
Gaussian variables							
pd	4.05	*0.06*	189	4.19	*0.09*	220	0.085
pd-L	10.48	*1.17*	8	8.09	*0.22*	17	0.009
	Median (s)	*range*[2]	n	Median (s)	*range*[2]	n	p
non Gaussian variables							
μpd	1.37	*1.36*	9	1.19	*1.44*	24	0.32
np	38.7	*215*	40	47.2	*152.2*	35	0.087
C	140.9	*1125*	49	176.4	*1195*	54	0.45

[1] Statistical tests performed: t test on Gaussian distributions (pd-L is almost Gaussian), Mann & Whitney test on all other variables; probability of test underlined when significant ($<$5%).
[2] range: *max* value – *min* value.

the mean time required by *A. gossypii* until the first cell was punctured (t-pd1) was twice as long for the non-infected plants as for the infected plants, but variability was high (p = 0.50). Aphids could lose the virus during longer extracellular penetrations, and therefore intracellular punctures immediately after the beginning of a probe could increase the efficiency of virus inoculation.

In spite of the mentioned correlation, not enough data were available for many variables and therefore, further experiments should be carried out to ascertain whether some other EPG variables (such as pd-L subphases . . .) are clearly and causally associated with CMV inoculation.

Discussion / conclusion

To our knowledge, this paper displays the first detailed analysis of individual potential drop durations in aphids. Also, the overall agreement between analyses from both experiments confirms the phenotypic identity of the two *Vat*-containing genotypes analysed. The between-experiment variability, although low, might result from either i) susceptible genotype background (Vedrantais vs Galia), ii) aphid genotype (clonal variability) or iii) experimental environment (differences in aphid pre-treatments or handling conditions). Therefore, comparing data between experiments must be carried out with extreme care, as these three factors may not be distinguished.

One of the questions that led to this work has clearly been answered: both aphid species, either sensitive or not to *Vat* as a virus vector (*A. gossypii vs M. persicae*), are able to detect the presence of the *Vat* gene in a very short period (i.e. less than 8 min of contact). The two species did not react similarly: both aphids reduced the individual duration of their intracellular punctures (both pd and pd-L), but the trend was higher and the reaction time was lower for *M. persicae* (–10% and detectable in 8 min, vs –8% and 20 min for *A. gossypii*). However, *A. gossypii*, but not *M. persicae*, added to this reaction a clear decrease in the frequency of intracellular punctures (pd) on the resistant genotype. The latter trait might be restricted to the early contact with resistant plant, since it was more pronounced in the 8-min experiment (–30%) than in the 20-min experiment (–22%), and may be influenced by short term adaptation of *A. gossypii* to probing on the resistant genotype, on which the phloem tissues are readily reached by this aphid (Chen et al., 1996b); however, the decrease in pd frequency was still detectable in a 2 h EPG experiment (Chen et al., 1996b).

Another interesting point was that recognition of the *Vat* genotype did not occur on first contact with the plant, revealing that both aphids displayed a 'reaction time' in this process. In none of the two experiments were the 'early variables' significantly discriminant (Tables 3 and 5). This could reflect the need for an integration of the chemical signal on which *Vat* resistance seems to be based, since active (deterrent) extractable factors were identified in the sap of resistant plants (Chen et al., 1996a). This is again in agreement with the altered pd duration on *Vat* plants, which needs some time to develop, especially with *A. gossypii*. Whether the product of the *Vat* gene is present intracellularly or extracellularly cannot be answered conclusively here, but the clear alteration of variables associated with cell punctures, together with the presence of deterrent activity in the phloem of resistant plants (Chen et al., 1996a) could be an indication of a symplastic expression of *Vat*. The other main question was whether the behavioural differences between *A. gossypii* and *M. persicae*, and of *A. gossypii* probing on R vs S genotypes, could account for the species-specific *Vat* effects on virus transmission. This question cannot be answered as clearly as the first one. The total resistance to CMV transmission by *A. gossypii* in our experimental conditions suggests the presence of extra-behavioural factors, as none of the differences observed here can explain such an absolute effect: i) all the described patterns have been identified in all of the 4 situations tested, and no obvious qualitative difference can be related to virus transmission, ii) although quantitative differences were detected (slight shortening of pd and decrease in pd frequency), it is biologically doubtful that they could lead to complete inhibition of transmission in the *A. gossypii* / R-plant combination, and, iii) none of the early variables that should be correlated with non-persistent virus transmission were significantly altered by the presence of the resistance gene.

If not behaviourally, *Vat* inhibition of viral transmission should therefore be chemically mediated, which is in agreement with the chemical determinism of the *antixenotic* response of *A. gossypii* to *Vat* plants (Chen et al., 1996a). Also, Powell et al. (1995) concluded in non-behavioural causes for the enhancement of potyvirus transmission by pre-acquisition starvation, which may implicate an unknown chemical mediation of non-persistent virus acquisition and release.

Nevertheless, rejecting the strict behavioural origin of *Vat* (anti-transmission) activity towards *A. gossypii* does not preclude all influence of feeding behaviour on virus transmission efficiency. As this experiment and others show (Lecoq et al., 1979; Fereres et al., 1992; Fereres et al., 1995), *M. persicae* is a much less efficient vector than *A. gossypii* for CMV inoculation. In reality, many behavioural traits differentiate the two aphids in both experiments, some of which may have a potential

influence in virus transmission. The most important of these traits is probably related to the structure and occurrence of pd-L, which was shorter (average 6 s vs 8 s) and much less stereotyped for *M. persicae* than for *A. gossypii*. In addition, pd-L almost always appeared as the first pd for *A. gossypii* (in 10–40 s), whereas for *M. persicae*, it only appeared after about 1 min and as the second or later pd. Bearing in mind the potential influence of pd-L length on inoculation efficiency (Table 6 and correlation analysis), this may partially explain the differences between the two species: *M. persicae* could lose part of its infectious load before the occurrence of an 'efficient' cell puncture. This may also apply to a lesser extent to *A. gossypii* on susceptible melons, where non-transmitting aphids spent slightly more time before a cell puncture than transmitter aphids (t-pd1 data, not shown; the same trend was detected with t-pd1 value for *A. gossypii* on the resistant genotype, Tables 3 and 5). These points emphasise the possible importance of pd-L in aphid vector biology, and may suggest a need for strict intracellular contact to allow any viral *inoculation*, as it was previously shown for viral *acquisition* (Lopez-Abella et al., 1988; Powell et al., 1995) and partially for potyvirus *inoculation* (Powell, 1991). The biological significance of such a special puncture is still unknown, but it apparently appeared on average only once during the global probe, regardless of its duration (unlike standard pd; Tables 3 and 5). Is pd-L histologically (epidermal cells?) or behaviourally determined ? Are there elementary behaviours (ingestion, egestion, salivation . . .) associated with its characteristic subphases? Finally, could it be functionally differentiated from other pds? (e.g. more devoted to host plant recognition, when shorter pds could better correspond to tissue recognition events?) These questions will remain open until specific experiments are conducted to define more clearly these long intracellular punctures.

Other traits, less related to virus transmission, do differentiate the two aphid species. *M. persicae* might be described as a more 'reactive' species than *A. gossypii*, as it reached the first phloem phase in less than 20 min, and detected the *Vat* gene product earlier, even though melon is not a host plant for this species. In Exp. 1, *M. persicae* also spent much more time probing (whatever the plant genotype, Table 3); this was not the case in Exp. 2 however (Table 5), and may reflect differences in aphid clones or pre-treatment between experiments (beans vs peppers, starvation time . . .), as do the differences in pd durations for *M. persicae* (–15% in Exp. 2 as compared to Exp. 1).

It must be noted that the callose-mediated interaction identified by Shinoda (1993) in another aphid-resistant melon genotype still has to be tested specifically in our *Vat* model. However, neither behavioural nor chemical indication for such an induced reaction was identified in the Margot line (Chen et al., 1996a, 1996b). In addition, the only cue to an interference with cell-wall/ pathway activities in our experiments was observed in Experiment 2 (total C duration significantly reduced in R, Table 5) but not in Experiment 1 (total C duration, Table 3); due to the different experiment durations, this might be caused by a lag in the development of a cell wall reaction. Nevertheless, the importance of the first minutes or even seconds of contact for non-persistent virus transmission makes it hard to rely on an induced, and therefore delayed, reaction for explaining *Vat* activity. Furthermore, no information is available on the susceptibility to virus inoculation of the melons used by Shinoda et al. (1993), and it has been shown that antixenosis and *Vat* phenotypes might be inherited independently in melon (Fereres et al., 1995), precluding further comparisons with our genetic material.

Finally, a tentative working hypothesis may be proposed for *Vat* activity on virus transmission: *Vat* is not able to inhibit virus uptake by *A. gossypii* in mechanically inoculated resistant plants (Lecoq et al., 1979; Fereres et al., 1995), and therefore acts only on the inoculation phase of many unrelated non-persistent viruses; thus, the *Vat* gene 'product' might be active through an inhibition of virus release (not specific of the virus), necessitating an interaction with some specific component from aphid origin (present in *A. gossypii* but not in *M. persicae*, either from stylet structural components or from salivary secretions). This hypothesis may be tested through the decomposition of the three partners of the interaction (plant, virus, aphid), and the *Vat* gene appears to be an interesting tool for an understanding of the specificities of the molecular interactions at the stylet tip. The fact that *Vat* is efficient on viruses differing widely in their transmission mechanisms (potyviruses, cucumoviruses . . .) may help to focus on the common components of such interactions. The interaction of stylet surface components with the virus-encoded 'bridge' macromolecules (helper components or capsid domains . . .) might be the target sites of such loosely specific phenomena related to non-persistent virus transmission, as the present *Vat* inhibition, or the well-known enhancement by pre-acquisition starvation (Powell, 1993; Wang and Pirone, 1996).

Acknowledgements

We would like to thank Hervé Lecoq and Michel Pitrat for useful comments on our manuscript, and Gérard Febvay for programming the EPG softwares. We are also grateful to Steve Murphy for the English corrections to our text.

References

Abacus Concepts (1989) Statview – SuperAnova; Abacus Concepts, Berkeley, CA

Chen JQ, Delobel B, Rahbé Y and Sauvion N (1996a) Biological and chemical characterisation of a genetic resistance of melon to the melon aphid. Entomol Exp Appl 80: 250–253

Chen JQ, Rahbé Y, Delobel B, Sauvion N and Febvay G (1996b) Melon resistance to the aphid *Aphis gossypii*: behavioural analysis and chemical correlations with nitrogenous compounds. Entomol Exp Appl in press

Collins JK, Perkins-Veazie P, Maness N and Cartwright B (1994) Resistance in muskmelon cultivars to melon aphid. Hortscience 29: 1367

Febvay G, Rahbé Y and Van Helden M (1996) MacStylet, software to analyse EPG (Electrical Penetration Graph) data on the Macintosh. Entomol Exp Appl 80: 105–108

Fereres A, Blua MJ and Perring TM (1992) Retention and transmission characteristics of zucchini yellow mosaic virus by *Aphis gossypii* and *Myzus persicae* (Homoptera: Aphididae). J Econ Entomol 85: 759–765

Fereres A, Martin B, Duque de Cela M and Alvarez JI (1995) Characterization of the resistance of melon lines to *Aphis gossypii* (Homoptera: Aphididae) and cucumber mosaic virus (CMV). In: Niemeyer H (ed) 12th Annual Meeting of the International Society of Chemical Ecology, 2–6 October, 1995, Santiago, Chile; ISCE

Fereres A, Shukle RH, Araya JE and Foster JE (1990) Probing and feeding behavior of *Sitobion avenae* (F.) (Hom. Aphididae) on three wheat cultivars infected with barley yellow dwarf virus. J Appl Entomol 109: 29–36

Garcia-Luque I, Kaper JM, Díaz-Ruiz JR and Rubio-Huertos M (1984) Emergence and characterization of satellite RNAs associated with Spanish cucumber mosaic virus isolates. J Gen Virol 65: 539–547

van Helden M and Tjallingii WF (1992) The use of Electrical Penetration Graphs in plant resistance research. In: Walker G and Backus E (eds) Proc. XIX Int. Congress of Entomology, Symp. on "Homopteran Feeding Behaviour: Recent Research advances and experimental techniques", Beijing, 07/1992; Thomas Say Publ.

Hodges LR and McLean DL (1971) Correlation of transmission of bean yellow mosaic virus with salivation activity of *Acyrthosiphon pisum* (Homoptera: Aphididae). Ann Entomol Soc Am 62: 1398–1401

Holbrook FR (1978) Transmission of potato leaf roll virus by the green peach aphid. Ann Entomol Soc Am 71: 830–831

Kennedy GG, McLean DL and Kinsey MG (1978) Probing behavior of *Aphis gossypii* on resistant and susceptible muskmelon. J Econ Entomol 71: 13–16

Khush GS and Brar DS (1991) Genetics of resistance to insects in crop plants. Adv Agron 45: 223–274

Kishaba AN, Bohn GW and Toba HH (1971) Resistance to *Aphis gossypii* in muskmelon. J Econ Entomol 64: 935–937

Kishaba AN, Castle SJ, Coudriet DL, McCreight JD and Bohn GW (1992) Virus transmission by *Aphis gossypii* Glover to Aphid-Resistant and susceptible muskmelons. J Am Soc Hort Sci 117: 248–254

Leclant F and Deguine JP (1994) Aphids (Hemiptera: Aphididae). In: Matthews G and Tunstall J (eds) Insect pests of Cotton (pp 285–323) CAB International, Oxon (GBR)

Lecoq H, Cohen S, Pitrat M and Labonne G (1979) Resistance to Cucumber Mosaic Virus transmission by aphids in *Cucumis melo*. Phytopathology 69: 1223–1225

Lecoq H, Labonne G and Pitrat M (1980) Specificity of resistance to virus transmission by aphids to *Cucumis melo*. Ann Phytopathol 12: 139–144

Lopez-Abella D, Bradley RHE and Harris KF (1988) Correlation between stylet paths made during superficial probing and the ability of aphids to transmit non-persistent viruses. Adv Dis Vect Res 5: 251–285

Lupoli R, Labonne G and Yvon M (1992) Variability in the transmission efficiency of potyviruses by different clones of *Aphis gossypii*. Entomol Exp Appl 65: 291–300

Mac Carter LE and Habeck DH (1973) The melon aphid: screening *Citrullus* varieties and introductions for resistance. J Econ Entomol 66: 1111–1112

McCreight JD, Kishaba AN and Bohn GW (1984) AR Hale's Best Jumbo, AR 5, and AR Topmark: Melon aphid-resistant muskmelon breeding lines. Hortscience 19: 309–310

McLean DL (1977) An electrical measurement system for studying aphid probing behavior. In: Harris KF and Maramorosch K (eds) Aphids as Virus Vectors (pp 277–290) Academic Press, New York (USA)

Perez P, Tjallingii WF and Fereres A (1996) Probing behaviour of *Myzus persicae* during transmission of Potato Virus Y to pepper and tobacco plants. Z Pflanzenk Pflanzen 103: 246–254

Pitrat M and Lecoq H (1980) Inheritance of resistance to cucumber mosaic virus transmission by *Aphis gossypii* in *Cucumis melo*. Phytopathology 70: 958–961

Pitrat M and Lecoq H (1982) Relations génétiques entre les résistances par non-acceptation et par antibiose du melon à *Aphis gossypii*. Recherche de liaisons avec d'autres gènes. Agronomie 2: 503–508

Powell G (1991) Cell membrane punctures during epidermal penetrations by aphids: consequences for the transmission of two potyviruses. Ann Appl Biol 119: 313–321

Powell G (1993) The effect of pre-acquisition starvation on aphid transmission of potyviruses during observed and electrically recorded stylet penetrations. Entomol Exp Appl 66: 255–260

Powell G, Harrington R and Spiller NJ (1992) Stylet activities and potato virus Y vector efficiencies by the aphids *Brachycaudus helicrysi* and *Drepanosiphum platanoidis*. Entomol Exp Appl 62: 293–300

Powell G, Pirone TP and Hardie J (1995) Aphid stylet activities during potyvirus acquisition from plants and *in vitro* system that correlate with subsequent transmission. Eur J Plant Pathol 101: 411–420

Prado E and Tjallingii WF (1994) Aphid activities during sieve element punctures. Entomol Exp Appl 72: 157–165

Scheller HV and Shukle RH (1986) Feeding behavior and transmission of barley yellow dwarf virus by *Sitobion avenae* on oats. Entomol Exp Appl 40: 189–195

Shinoda T (1993) Callose reaction induced in melon leaves by feeding of melon aphid, *Aphis gossypii* Glover as possible Aphid-Resistant factor. Jpn J Appl Entomol Zool 37: 145–152

Shinoda T and Tanaka K (1989) Resistance of melon, *Cucumis melo* L. to the melon aphid, *Aphis gossypii* Glover. II Distribution of the behaviour of aphids on resistant and susceptible melon leaves. Jpn J Appl Entomol Zool 33: 249–251

Shukle RH, Lampe DJ, Lister RM and Foster JE (1987) Aphid feeding behavior: relationship to barley yellow dwarf virus resistance in *Agropyron* species. Phytopathology 77: 725–729

Simons JN, McLean DL and Kinsey MG (1977) Effects of mineral oil on probing behavior and transmission of stylet-borne viruses by *Myzus persicae*. J Econ Entomol 70: 309–315

Tjallingii WF (1978) Electronic recording of penetration behaviour by aphids. Entomol Exp Appl 24: 521–530

Tjallingii WF (1988) Electrical recording of stylet penetration activities. In: Minks AK and Harrewijn P (eds) Aphids, their Biology, Natural enemies and Control. Vol B (pp 95–108) Elsevier, Amsterdam

Wang RY and Pirone TP (1996) Potyvirus transmission is not increased by pre-acquisition fasting of aphids reared on artificial diet. J Gen Virol 77: 3145–3148