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Assessment of transmission ability of barley yellow dwarf virus-PAV isolates by different populations of *Rhopalosiphum padi* and *Sitobion avenae*

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Abstract

Populations of two aphid species from different geographic regions of Morocco were tested for their ability to transmit five barley yellow dwarf virus-PAV (BYDV-PAV) type isolates using Clintland 64 oat as the test plant. Transmission efficiencies were determined for 10 sub-populations of *Rhopalosiphum padi* and 12 sub-populations of *Sitobion avenae*. After a short acquisition access period (AAP) of 4 h, all populations transmitted the virus but with different efficiencies. *R. padi* (Rp-S) and *S. avenae* (Sa-S) collected in the Settat region were the most efficient vectors, with transmission rates of 38% and 27%, respectively. *R. padi* (Rp-C) collected at Chaouen and *S. avenae* (Sa-B) at Berkane, were poor transmitters with respective vectoring abilities of 20% and 16%. These four sub-populations were chosen to study the acquisition of BYDV-PAV and the retention of virus within aphids in more detail. The transmission after two AAPs of 4 h and 48 h were compared. Starved aphids given a 4 h AAP had significantly higher transmission efficiencies than non-starved aphids. However, after a 48 h AAP, no difference was observed in the transmission between starved and non-starved aphids. Intraspecific variability was also detected by means of serial transfers of individual aphids after the given AAP. Following the first day of serial transfers, no differences were observed in transmission efficiency and virus titers for sub-populations within each species acquiring the virus during 48 h, but there was significant variation when the virus was acquired in 4 h. The levels of PAV antigen retained by aphids fed on healthy plants declined rapidly during the first day after acquisition, but remained fairly constant during the next 5–7 days depending on the length of the AAP. Virus antigen could be detected by ELISA in Rp-S and Sa-S for up to 11 days of serial transfer, but it was shown that aphids could retain and transmit BYDV-PAV for at least 3 weeks.

Introduction

Barley yellow dwarf (BYD) is an important viral disease. The virus (BYDV) has a wide host range including all of the major cereal crops and many annual and perennial weeds, pasture and range grasses (Lister and Ranieri, 1995). BYDV is vectored by several aphid species in a persistent circulative manner and is mainly limited to the phloem tissue of an infected plant (Gray, 1996). The virus has 25 nm icosahedral particles with a single-stranded, monopartite, positive-sense RNA genome which lacks a terminal VPg (Miller and Rasochova, 1997). BYDV occurs as a diverse complex of at least five species that vary greatly in aphid transmissibility and severity in cereal hosts (Rochow, 1969). The species RPV, RMV, MAV and SGV are readily transmitted by *Rhopalosiphum padi* (L.), *R. maïdis* (Fitch), *Sitobion* (formely *Macrosiphum*) *avenae* (Fabricus), *Schizaphis graminum* (Rondani), respectively, while PAV is transmitted by both *R. padi*

and *S. avenae* (Rochow, 1969; Johnston and Rochow, 1972; Rochow and Muller, 1971; Power et al., 1991).

The BYDV species have been split into two subgroups, I and II, based upon cytopathological ultrastructure (Gill and Chong, 1979), serological relatedness (Rochow and Carmichael, 1979), genome organization and sequence divergence (Waterhouse et al., 1986; Vincent et al., 1990; Miller and Rasochova, 1997). Subgroup I contains PAV, MAV and SGV, and subgroup II contains RPV and RMV (Murphy et al., 1995). Recently, D'Arcy and Mayo (1997) proposed a new classification in which a new family *Luteoviridae* replaced the *Luteovirus* genus. In this new classification, BYDV-PAV and BYDV-MAV were assigned to the *Luteovirus* genus, and the former RPV was named cereal yellow dwarf virus-RPV (CYDV-RPV) and was assigned to the *Polerovirus* genus. In this proposal, BYDV-SGV and BYDV-RMV were classified as unassigned viruses in the family *Luteoviridae*. This classification has now been accepted by the International Committee on Taxonomy of Viruses (ICTV) and the new nomenclature will be used throughout this paper (D'Arcy et al., 1999).

The spread of persistently transmitted plant viruses depends on vector propensity, which is the probability that a vector will successfully inoculate a plant with a virus (Irwin and Ruesink, 1986). The acquired virus particles are transported through gut epithelial cells and eventually arrive in the accessory salivary glands (Gildow, 1993; Gildow and Gray, 1993). Virus–aphid specificity seems to result from recognition between virions of a specific isolate and virus receptors in the accessory salivary glands of a particular aphid species (Gildow and Rochow, 1980; Gildow and Gray, 1993; Peiffer et al., 1997). It is well documented that the various aphid species differ in their abilities to transmit the various variants of BYDV (Rochow, 1969; Rochow and Carmichael, 1979). However, little is known about intraspecific aphid variation. Rochow (1960) reported specialization among clones of *S. graminum* collected in the USA in the transmission of SGV isolates. Variations in transmission of PAV and MAV variants were also described in France (Guo et al., 1997a,b; Sadeghi et al., 1997a,b). In Morocco, the spread of BYDV-PAV depends mainly on transmission by *R. padi* and *S. avenae* (El Yamani and Hill, 1991; El Yamani and Bencharki, 1997). In this study, we sought to determine if Moroccan BYDV-PAV isolates, originating from different regions and differing in coat protein amino acid sequences and virulence, differ also in their transmissibility efficiency and retention (persistence) among several sub-populations of *R. padi* and *S. avenae*.

Materials and methods

Culture of virus isolates

BYDV-PAV type isolates used in this study are listed in Table 1. The geographic and host origins, and the biological and molecular properties of those isolates have been described (Bencharki et al., 1999). The isolates were maintained by repeated aphid transfers on 7-day-old seedlings of Clintland 64 oat at intervals of 5–6 weeks. The plants were inoculated with viruliferous *R. padi* nymphs with a 3 day inoculation access period (IAP). After inoculation, plants were kept in a growth chamber (Rheem, Model CEE 38-15 HLE, USA) at $18 \pm 2\,^{\circ}$C with 16 h illumination per day for symptoms to develop. Young leaves of the same age from infected Clintland 64 plants, 3 weeks after the IAP, were used as a source of the virus in all experiments.

Aphid clone maintenance

Aphid colonies were collected during the cropping seasons of 1994–95 in different regions in Morocco. In total, 12 populations of *S. avenae* (from Settat, Khenifra, Ouled Frej, Oujda, Deroua, Meknes, Azrou, Safi, Nador, Berkane, Guercif, Chaouen) and 10 of *R. padi* (from Settat, Youssofia, Aghbala, Meknes, Nador, Berkane, Deroua, Taourirt, Ifrane, Chaouen) were sampled from a single cereal host-plant (barley, oat, durum or bread wheat). A few aphids were collected for each population. Colonies of each population originated from a nymph born from a single

Table 1. List of barley yellow dwarf virus PAV isolates used in this study

BYDV-PAV isolates	Geographical origins	Symptom severity	Cluster[a]
MA9415	Settat	Severe	II
MA9502	Youssofia	Mild	I
MA9513	Taza	Mild	I
MA9514	Guercif	Severe	II
MA9517	Aghbala	Severe	II

[a]Classification based on the amino acid sequence of the coat protein gene (see Bencharki et al., 1999).

viviparous aphid on filter paper. Virus-free colonies of the collected aphids were maintained as described by El Yamani and Hill (1991). These colonies were grown on caged Clintland 64 oat plants in a greenhouse compartment at $18 \pm 2\,^{\circ}C$, with precautions to prevent any accidental mixture between sub-populations and contamination by BYDV. Since each sub-population originates from an individual aphid, it may be expected that its competence to transmit will not differ from its parent. For continuous maintenance of the sub-populations, one aphid adult was transferred to a new healthy Clintland 64 seedling every 3 weeks. All transmission studies were done with 2- to 3-day-old apterous adults caged separately on Clintland 64 oat seedlings.

Testing for virus-transmission efficiency

Virus-free aphids of each sub-population were removed from the rearing oat plants and given a short 4 h acquisition access period (AAP) on detached leaves from an oat plant infected with the PAV isolate studied. Aphids were then caged individually on 7-day-old Clintland 64 oat seedlings for 72 h IAP. Fifteen test seedlings with a single aphid per plant were used, and the entire experiment was repeated three times. At the end of the IAP, plants were sprayed with the insecticide deltametrine (2.5 a.i. g/l; AgrEvo, France) and maintained in an insect-proof greenhouse. Uninfected Clintland 64 plants were grown in the same conditions. Disease symptoms were scored 4 weeks after inoculation.

Effect of prior starvation on aphid transmission

From the preliminary transmission tests, two sub-populations of *R. padi* and of *S. avenae* species were chosen to analyze their potential as vectors more thoroughly. These sub-populations were the more efficient transmitters, i.e., *R. padi*-Settat (hereafter Rp-S) and *S. avenae*-Settat (hereafter Sa-S) and the less efficient transmitters, i.e., *R. padi*-Chaouen (hereafter Rp-C) and *S. avenae*-Berkane (hereafter Sa-B). All aphids were either starved or allowed to feed normally before the AAP. The starved aphids were kept in a Petri dish overnight at room temperature before the experiment, while the non-starved aphids were collected from the rearing plant just before the AAP. Transmission tests were done using one aphid per plant after a short (4 h) or a long (48 h) AAP on infected leaves. To test each combination, 18 aphids were used and each test was repeated three times.

Persistence and retention of PAV by its aphid vectors

After a 4 or 48 h AAP, 18 aphids of each sub-population were placed individually on caged 7-day-old Clintland 64 oat seedlings and serially transferred for 24 h IAP to test plants, until all of the aphids were dead. As a control, aphids that had not fed on a source plant were serially transferred to test plants. The plants were kept under greenhouse conditions at $18 \pm 2\,^{\circ}C$ with a photoperiod of 16 h day-length for symptoms to develop.

Another experiment was done to compare virus transmission and persistence of virus after successive transfers. Aphids were given an AAP of 4 or 48 h on infected oat plants. Groups of 12 aphids of each sub-population were removed ensuring a population of the same age from plants using a camel hair brush at 2 day intervals, during 15 days of serial transfer. Individual aphids were homogenized or stored at $-20\,^{\circ}C$ to be assayed for their virus content by ELISA.

Virus detection

The virus content of plant and aphid extracts was determined by double antibody sandwich-ELISA (DAS-ELISA) in microtiter plates (ImmulonI, Dynatech Laboratories, Inc. Alexandria, VA) as described by El Yamani and Hill (1990) with minor modifications. The BYDV-PAV (strain B) polyclonal antiserum used for DAS-ELISA was purchased from Inotech (Basel, Switzerland). The background observed in healthy aphid extracts was reduced by blocking the plates for 4 h with 0.1% bovine serum albumin in $1\times$ PBS buffer, pH 7.4. Samples that gave readings higher than the average reading from healthy controls plus three standard deviations were scored as positive; those with lower readings were considered negative.

Results

Vector efficiency of different aphid sub-populations

Previous studies on the transmissibility of BYDV-PAV isolates by a sub-population of *R. padi* and *S. avenae*

were extended to several sub-populations of both aphid species (El Yamani and Bencharki, 1997). Results of vector transmission experiments with different PAV isolates revealed a significant variation between BYDV-PAV and *R. padi* sub-populations, and BYDV-PAV and *S. avenae* sub-populations ($P < 0.01\%$) (Tables 2 and 3). This suggests that the transmission efficiency of the different *R. padi* and *S. avenae* sub-populations is dependent upon the PAV isolate.

The average of the transmission rates showed that the interaction between virus isolates and aphids is significant ($P < 0.01\%$). All sub-populations of *R. padi* and *S. avenae* could transmit the five BYDV-PAV isolates after a 4 h AAP, but with different transmission efficiencies. The average percentage transmission varied from 20% for Rp-C to 38% for Rp-S sub-populations (Table 2) and from 15% for Sa-B to 27% for Sa-S sub-populations (Table 3). The highly efficient vectors Rp-S and Sa-S transmitted the virus two times better than the less efficient vectors, Rp-C and Sa-B.

The transmissibility of BYDV-PAV isolates by different *R. padi* and *S. avenae* aphid sub-populations was also examined. When averaging the percentage of transmission by all sub-populations among species, significant differences ($P < 0.01$) in transmissibility of PAV isolates was found (Tables 2 and 3). In particular, isolate MA9517 was more efficiently transmitted compared to the other isolates.

Effect of pre-acquisition starvation on aphid competence

In order to investigate the relation of the virus and the vectors, *R. padi* and *S. avenae*, the transmission of the highly transmissible isolate MA9517 was studied with both starved and non-starved aphids. The more efficient Rp-S and Sa-S and the less efficient Rp-C and Sa-B transmitters were used in this study. The aphids were individually tested after a 4 or 48 h AAP. With a short AAP (4 h), starved aphids transmitted BYDV-PAV more efficiently than non-starved aphids (Table 4). Starvation increases the transmission ability by 22%, 21%, 33%, and 27% for Rp-S, Rp-C, Sa-S and Sa-B, respectively. In contrast, when the AAP was 48 h, no significant differences were found between starved and non-starved aphids. It is important to note that, compared to the starved aphids, the non-starved aphids took a longer time to explore the leaf surface before punctation and probing. This demonstrates that transmission efficiency is related to feeding behavior/uptake and so is the amount of virus acquired and transmitted.

Retention of BYDV-PAV isolate with its vectors

In these experiments, starved aphids of each sub-population were given an AAP of 4 or 48 h. Aphids were then individually transferred to healthy 7-day-old seedlings plants every 24 h of IAP. Eighteen aphids were used to test each combination of virus, vector and AAP. The results of these serial transfers of single aphids showed that Rp-S transmitted this virus with an efficiency of 47% and 97% after a 4 or 48 h AAP, respectively, and that Rp-C had values of 31% and 86% in the first day after the AAPs, but diminished efficiency following successive transfers (Figure 1a and b). The period over wich transmission can occur (i.e., persistence of transmission) depended on the length of the acquisition period. After a 4 h AAP, Rp-C and Rp-S transmitted the virus for 10 and 14 days, and Sa-B and Sa-S for 8 and 13 days, respectively. After 48 h AAP, the aphids were able to transmit the virus throughout almost the entire test period of 3 weeks. The transmission rate found for both sub-populations was significantly different throughout the entire period of serial transfers. However, after 48 h AAP, slight differences in transmission efficiency between Rp-S and Rp-C and between Sa-S and Sa-B sub-populations were detected during the first 2 days of the experiment.

A parallel experiment was conducted to quantitatively compare virus transmission and aphid virus content after serial transfers. Groups of 12 aphids were removed ensuring a population of same age, and assayed individually for their virus content with ELISA. Detection of PAV in single aphids by ELISA was improved by applying a long blocking step (4 h) with 0.1% BSA, which slightly reduced the background signal for non-viruliferous aphids. Figure 2 shows that no differences in the amount of virus could be detected in the aphids of the four *R. padi* and *S. avenae* sub-populations immediately after 48 h AAP, whereas the amount of virus acquired after 4 h differed significantly. The $A_{410\,nm}$ values after 48 h AAP were markedly higher than those after 4 h AAP. Thus, after 48 h AAP, the average $A_{410\,nm}$ values are 0.42, 0.40, 0.39, and 0.36 for Rp-S, Rp-C, Sa-S and Sa-B, respectively, and 0.30, 0.22, 0.28, and 0.20 after the 4 h AAP.

Studying the virus in individual aphids, virus was detected in 83% and 100% of individual Rp-S, 67% and

Table 2. Comparison of transmission efficiency between *Rhopalosiphum padi* sub-populations for different BYDV-PAV isolates

Sub-populations		Transmission rates of BYDV-PAV isolates[a]						
Location of collection	Names	MA9517 Aghbala	MA9513 Taza	MA9415 Settat	MA9514 Guercif	MA9502 Youssofia	Means[d] (SE)	LSD[c]
Settat	Rp-S	39.5[b] ± 1.3	39.5 ± 1.3	40.6 ± 0.0	35.3 ± 3.6	36.3 ± 0.6	38.2 ± 2.1	3.9
Youssofia	Rp-Y	39.8 ± 0.3	37.8 ± 2.0	37.8 ± 3.3	30.8 ± 1.4	34.5 ± 4.0	36.1 ± 3.2	4.9
Aghbala	Rp-A	34.5 ± 0.5	31.6 ± 0.0	35.8 ± 1.6	35.8 ± 1.7	32.8 ± 1.4	34.1 ± 1.7	2.6
Meknes	Rp-M	39.5 ± 0.9	35.8 ± 2.0	35.8 ± 1.4	30.8 ± 2.3	22.6 ± 0.0	32.9 ± 5.9	2.5
Nador	Rp-N	30.6 ± 2.1	31.6 ± 0.0	27.6 ± 1.0	27.7 ± 0.0	22.6 ± 0.0	28.0 ± 3. 1	2.0
Berkane	Rp-B	39.7 ± 3.1	29.3 ± 0.6	21.3 ± 2.1	21.3 ± 1.7	24.8 ± 1.4	27.2 ± 6 9	3.9
Deroua	Rp-D	36.4 ± 2.6	29.3 ± 0.6	26.1 ± 0.0	27.6 ± 1.0	17.8 ± 2.7	27.4 ± 6.0	3.4
Taourirt	Rp-T	34.0 ± 4.0	26.1 ± 2.0	27.6 ± 0.0	20.3 ± 0.0	18.3 ± 1.5	25.2 ± 5.6	4.4
Ifrane	Rp-I	31.3 ± 2.1	26.1 ± 0.2	21.1 ± 3.1	19.1 ± 0.0	15.8 ± 3.3	22.5 ± 5.6	4.1
Chaouen	Rp-C	25.2 ± 3.0	17.6 ± 0.0	21.1 ± 1.0	20.3 ± 0.6	15.8 ± 3.0	20.0 ± 3.2	3.8
LSD[c]		4.0	1.8	3.2	3.0	3.8	1.4	
Means[d] (SE)		35.1 ± 4.7	30.5 ± 6.1	29.4 ± 7.1	26.9 ± 6.0	24.1 ± 7.5		

[a]Data are means of three replicates, each involving 15 plants for the different BYDV-PAV isolates. No significant differences in percentage of transmission occurred between replicates. Aphids were given 4 h acquisition access period feeding on detached leaves from infected Clintland 64 oats. Single aphids were then allowed a 3 day IAP feeding on 7-day-old Clintland 64 oats. None of the aphids of any sub-population transmitted the virus when fed on healthy plants.
[b]Values are the means of three replicates of 15 plants.
[c]Means within a column for BYDV-PAV isolates and means within a row for aphid sub-populations were compared using Fisher's least significant difference (LSD) at $\alpha = 0.05$.
[d]Means and their standard errors (SE).

Table 3. Comparison of transmission efficiency between *Sitobion avenae* sub-populations for different BYDV-PAV isolates

Sub-populations		Transmission rates of BYDV-PAV isolates[a]						
Location of collection	Names	MA9517 Aghbala	MA9415 Settat	MA9513 Taza	MA9514 Guercif	MA9502 Youssofia	Means[d] (SE)	LSD[c]
Settat	Sa-S	30.8[b] ± 2.4	26.2 ± 0.0	27.8 ± 1.4	26.2 ± 2.0	22.5 ± 2.2	26.7 ± 2.7	3.4
Kenifra	Sa-K	25.3 ± 0.0	23.3 ± 3.1	22.5 ± 1.8	23.7 ± 4.5	22.5 ± 2.2	23.4 ± 1.0	4.9
Ouled Frej	Sa-F	25.3 ± 0.0	20.0 ± 5.3	28.7 ± 0.0	23.3 ± 3.1	20.0 ± 5.2	23.4 ± 3.3	6.8
Oujda	Sa-O	25.7 ± 1.6	27.7 ± 2.3	28.0 ± 4.0	22.1 ± 3.6	17.7 ± 0.0	24.2 ± 3.9	3.5
Deroua	Sa-D	27.7 ± 3.2	26.2 ± 2.0	27.0 ± 2.1	19.8 ± 1.4	19.5 ± 1.8	24.2 ± 3.8	4.4
Meknes	Sa-M	25.3 ± 2.5	20.1 ± 3.4	21.2 ± 0.0	19.0 ± 4.2	15.6 ± 3.2	20.2 ± 3.1	5.4
Azrou	Sa-A	22.8 ± 3.3	22.8 ± 0.0	23.3 ± 4.6	14.3 ± 0.0	11.2 ± 1.9	18.9 ± 5.1	4.1
Safi	Sa-Sf	21.2 ± 0.0	20.6 ± 2.9	17.5 ± 2.9	15.2 ± 3.0	16.2 ± 4.3	18.1 ± 2.4	5.9
Nador	Sa-N	20.0 ± 4.0	20.0 ± 5.3	17.0 ± 2.6	16.3 ± 4.0	13.5 ± 4.5	17.3 ± 2.5	7.1
Berkane	Sa-B	19.3 ± 2.9	13.1 ± 3.6	19.4 ± 2.1	11.3 ± 0.6	10.3 ± 4.0	14.6 ± 3.9	5.1
Guercif	Sa-G	16.7 ± 0.0	15.1 ± 3.4	20.7 ± 3.1	15.0 ± 3.0	14.2 ± 0.0	16.3 ± 2.3	4.9
Chaouen	Sa-C	17.8 ± 2.4	16.3 ± 4.0	18.0 ± 5.0	13.3 ± 3.1	14.8 ± 3.3	16.0 ± 1.8	7.7
LSD[c]		6.0	5.9	4.9	5.2	5.5		
Means[d] (SE)		23.1 ± 4.1	20.9 ± 4.4	22.6 ± 4.2	18.3 ± 4.5	16.5 ± 3.9		

[a]Data are means of two experiments of three replicates, each involving 15 plants for the different BYDV-PAV isolates. No significant differences in percentage of transmission occurred between replicates. Aphids were given 4 h acquisition access period feeding on detached leaves from infected Clintland 64 oats. Single aphids were then allowed a 3 day IAP feeding on 7-day-old Clintland 64 oats. None of the aphids of any sub-population transmitted the virus when fed on healthy plants.
[b]Values are the means of three replicates of 15 plants.
[c]Means within a column for BYDV-PAV isolates and means within a row for aphid sub-populations were compared using Fisher's LSD at $\alpha = 0.05$.
[d]Means and their standard errors (SE).

Table 4. Transmission efficiency of BYDV-PAV by starved and non-starved *R. padi* and *S. avenae* sub-populations

Aphid sub-populations[a]	No. of aphids (of 54) that transmitted the BYDV-PAV after the AAP[b]			
	Non-starved aphids		Starved aphids	
	4 h	48 h	4 h	48 h
Rp-S	21 (39%)	50 (93%)	28 (51%)	52 (96%)
Rp-C	14 (26%)	46 (85%)	17 (32%)	48 (89%)
Sa-S	15 (28%)	32 (59%)	20 (37%)	34 (63%)
Sa-B	08 (15%)	28 (52%)	11 (20%)	30 (56%)

[a]Rp-S and Rp-C indicate sub-populations of *R. padi* collected at Settat and Chaouen, respectively. Sa-S and Sa-B indicate sub-populations collected at Settat and Berkane, respectively.
[b]Data are combined results of three experiments, each involving 18 plants that used BYDV-PAV MA9517 isolate collected at Aghbala. No significant differences occurred in percentage of transmission between experiments. Aphids were given acquisition access periods of 4 h or 48 h feeding on detached leaves from infected Clintland 64 oats. Single aphids were then allowed a 3 day IAP feeding on 7-day-old Clintland 64 oats. None of the aphids transmitted the virus when fed on healthy plants.

92% for Rp-C, 67% and 92%, for Sa-S 58% and 83% for Sa-B aphids tested after 4 and 48 h AAP, respectively (Table 5). Throughout the successive daily transfers of aphids to fresh plants, $A_{410\,nm}$ values decreased and the number of aphids in which no virus could be detected increased. After 5 days, virus was undetectable in 33%, 58%, 58% and 75% of the Rp-S, Rp-C, Sa-S and Sa-B aphids. Even, when virus could not longer be detected by ELISA, aphids remained sometimes infectious during their lifetime. However, the plants inoculated with older aphids displayed delayed symptom expression compared with plants inoculated with younger aphids. All the aphids were dead after 27 days of successive transfers.

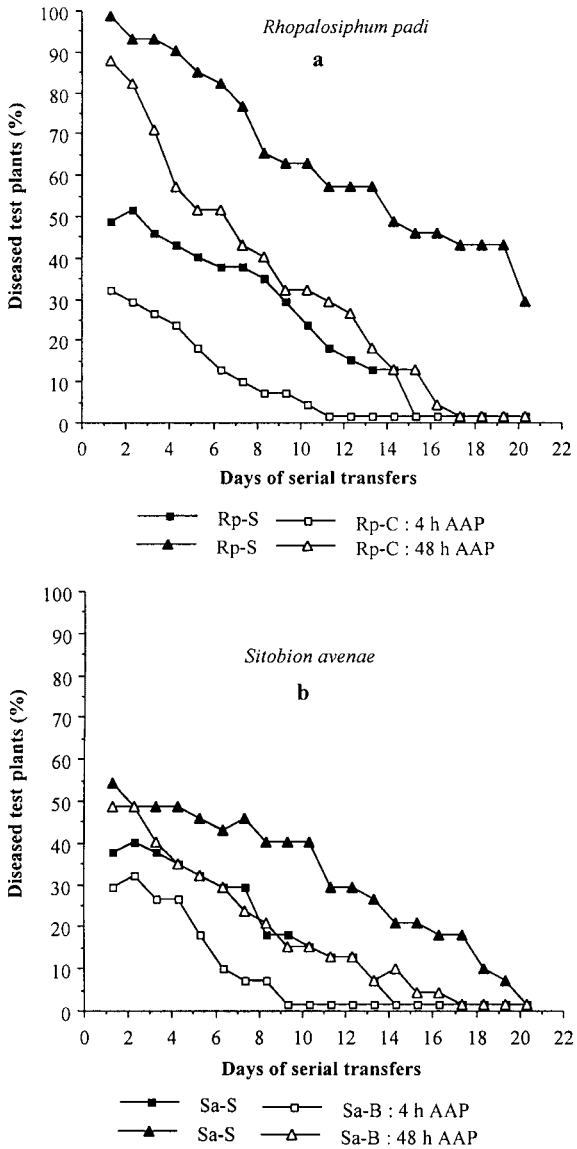

Figure 1. Differential transmission efficiency for a succession of serial transfers of MA9517 isolate of BYDV-PAV by two sub-populations of *R. padi* (Rp-S and Rp-C) and two sub-populations of *S. avenae* (Sa-S and Sa-B), after 4 or 48 h AAP. Individual aphids were transferred daily on healthy 7-day-old Clintland 64 oat plants until all the aphids died. Symptoms were recorded after 4 weeks incubation.

Discussion

For a reliable comparison of sub-populations of *R. padi* and *S. avenae* with respect to virus transmission efficiency, experiments were performed using previously characterized BYDV-PAV isolates (Bencharki et al., 1999). Both the previous work and this report substantiate that *R. padi* is the most efficient/competent vector of BYDV-PAV (Rochow, 1969; El Yamani and Hill, 1991; Gray et al., 1991; Power et al., 1991). Moreover, the current results indicate that there is significant intraspecific variation in aphid transmission of BYDV-PAV isolates between *R. padi* and *S. avenae*. The transmission rates varied from 20% to 38% for *R. padi* and from 16% to 27% for *S. avenae* sub-populations. The occurrence of intraspecific aphid variation described here is not an isolated example. Differences in transmission were also found for *R. padi* and *S. avenae* in Western Europe (Guo et al., 1997a,b; Sadeghi et al., 1997a) and for *S. avenae* in the USA (Saksena et al., 1964;

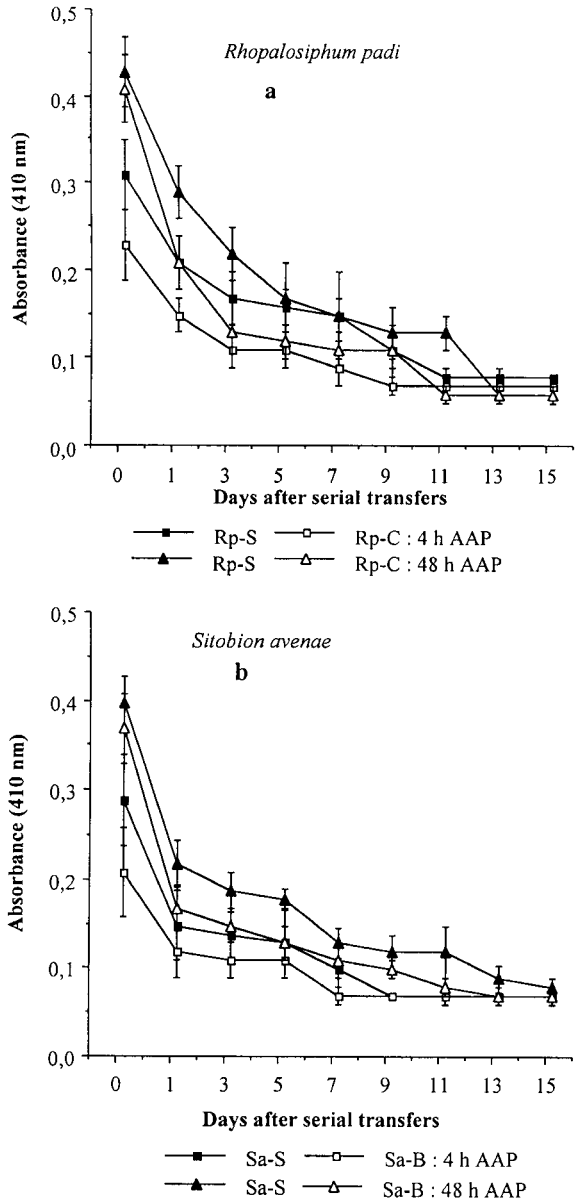

Figure 2. Sequential measurements of virus titer in single aphids (absorbance at 410 nm) of MA9517 isolate of BYDV-PAV in single aphids of *R. padi* (Rp-S and Rp-C) and two sub-populations of *S. avenae* (Sa-S and Sa-B) after 4 and 48 h AAP, during a succession of serial transfers. For each sub-population, 12 aphids were sampled at 2 day intervals during 15 days of the serial transfers on healthy 7-day-old Clintland 64 oat plants. The virus titer in different aphid sub-populations was analyzed by ELISA.

Rochow and Eastop, 1966; Gildow and Rochow, 1983; Lei et al., 1995). However, the current results do not support the findings of Gildow and Rochow (1983) and Guo et al. (1997b). These authors reported no

Table 5. Persistence of BYDV-PAV in sub-populations of *R. padi* and *S. avenae* during successive transfers after different AAP

Days of serial transfer[b]	No. of aphids (of 12) with detectable BYDV-PAV							
	Rp-S[a]		Rp-C		Sa-S		Sa-B	
	4 h[c]	48 h	4 h	48 h	4 h	48 h	4 h	48 h
0	10[d]	12	11	9	8	11	7	9
1	8	12	10	7	7	9	4	7
3	8	12	9	5	7	9	4	6
5	8	11	5	5	5	5	3	4
7	6	12	0	3	4	4	0	5
9	0	7	0	0	0	5	0	0
11	0	6	0	0	0	5	0	0
13	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0

[a]Rp-S, Rp-C indicates sub-populations of *R. padi* collected at Settat and Chaouen, respectively. Sa-S and Sa-B indicates sub-populations collected at Settat and Berkane, respectively.

[b]Sampled aphids were assayed individually for BYDV-PAV antigen by DAS-ELISA at 2 day intervals following daily successive transfers on healthy Clintland 64 plants after 4 h or 48 h AAP.

[c]AAP.

[d]Each result shows the number of aphids with detectable BYDV-PAV as determined by ELISA tests. Positive threshold values equaled the mean absorbance values of 3 aphids maintained on healthy Clintland 64 plants plus three standard deviations.

differences in the ability to transmit NY-PAV and CA-PAV, and CYDV isolates for one New York and two California *R. padi* clones, after a long acquisition feeding period (48 h). However, Guo et al. (1997a) observed significant variation in vector efficiency in PAV transmission by *R. padi* and *S. avenae*, when using a 5-day-AAP. The results, obtained in experiments when aphids were allowed to acquire virus in 4 or 48 h on comparable virus sources, suggest that transmission of BYDV depends on the virus dose acquired. The longer the AAP, the higher was the percentage of transmitting aphids. These observations could be related to differences in vector competence.

In this paper, we have demonstrated diversity in transmissibility for the Moroccan BYDV-PAV isolates. These five isolates were previously grouped into two clusters on the basis of virulence and coat protein gene sequences (Table 1) (Bencharki et al., 1999). Isolate MA9513, which showed mild symptoms on barley and oat, was efficiently transmitted with average transmission rates by *R. padi* (31%) and *S. avenae* (21%). However, isolate MA9514 which produced

severe symptoms, was transmitted at rates of 27% and 18% by *R. padi* and *S. avenae*, respectively. The other isolates also show a positive correlation between virulence and transmissibility. Eweida et al. (1988) found that BYDV occurred in aggregated forms in the phloem cells in oats. The association between virus and membrane constituents may explain the availability of virus for acquisition by aphids. Peters and Elderson (1984), and Van den Heuvel et al. (1993) reported a lower potato leafroll virus (PLRV) transmission efficiencies by *Myzus persicae* from infected *Physalis floridana* plants showing severe symptoms than from plants with mild symptoms. These observations suggest that the amount of virus in the cells decreased with symptom severity. Therefore, availability of acquirable virus could differ with the virulence of the isolate. Note that the observed diversity in transmission efficiencies in our experiments cannot be explained by differences in the virus concentration because the source leaves showed similar absorbance values (data not shown). However, it has been suggested that the reduced levels of transmission of BYDV-PAV, -MAV, -RMV and -SGV were correlated with a reduction in virus concentration (Gray et al., 1993).

The diversity in transmission ability could also be related to differences in the surface of the viral capsid of the different isolates. Gildow and Rochow (1983) suggested that the capsid is often the main source causing variations in the virus–vector interactions. Using immunological studies, Van den Heuvel et al. (1993) revealed that two phenotypic variants of PLRV that differ in transmissibility by *M. persicae*, differ in capsid integrity. In our experiments, differences in vector efficiency were measured as accurately as possible by using high numbers of aphids. We suggest that the observed intraspecific variability in efficiency of transmission may be related, at least in part, to differences in ability of the movement of BYDV-PAV virus throughout the epithelial cell barriers at the hindgut and/or the accessory salivary glands in the different clones (Gildow, 1993; Gildow and Gray, 1993) or to the stability of the virus in the hemolymph (Van den Heuvel et al., 1997).

Efficiency of virus transmission is generally increased by pre-acquisition starvation of the aphids for non-persistently transmitted viruses (Nault, 1997; Pirone and Harris, 1977; Wang and Pirone, 1996). Powell (1993) reported that pre-acquisition starvation affects aphid behavior, causing stylet penetration to occur earlier than for non-starved aphids, with a consequent increase in potyvirus transmission. Our studies revealed that the transmissibility of BYDV is also increased by pre-acquisition starvation. The effect of starvation on BYDV transmission can be expressed as the quotient of transmission rates of starved versus non-starved aphids. The quotient is similar for *R. padi* sub-populations Rp-S (22%) and Rp-C (21%), but it is quite different for *S. avenae* sub-populations Sa-S (33%) and Sa-B (27%). This finding is consistent with the idea that the enhancement of virus transmission by pre-acquisition starvation is determined by aphid behavioral factors. A previous study which compared feeding behavior of *R. padi* and *S. avenae* by using electronic monitoring demonstrated that *S. avenae* spend more than twice as long in salivation and sheath formation than *R. padi* and is only half as long in contact with the phloem (Shukle et al., 1987).

It should be noted that when using starved aphids in daily successive transfers, BYDV-PAV is detected by ELISA in individual aphids only during the first 7 and 11 days after 4 and 48 h AAPs, respectively (Table 5). The virus titer decreased with time. However, the decrease of virus content in the four sub-populations tested was also AAP-dependent. After a 48 h AAP, similar average ELISA ($A_{410\,nm}$) values were detected for *R. padi* sub-populations Rp-S (0.42) and Rp-C (0.40), and *S. avenae* sub-populations for Sa-S (0.39) Sa-B (0.36), following the first day of serial transfers (Figure 2). Then, the values decreased rapidly in aphids and reached a constant level after 5–7 days depending on the aphid sub-population and AAP. Van den Heuvel et al. (1994) have demonstrated that symbionin, a GroEL homologous protein synthesized by endosymbiotic bacteria (genus *Buchnera*) and secreted into the aphid hemolymph, is essential for efficient luteovirus transmission. Possibly, symbionin of the inefficient aphid sub-populations Rp-C and Sa-B is released at low concentrations or has low binding affinity for the virus. The later possibility is indirectly supported by the recent findings of Van den Heuvel et al. (1997), who showed that symbionin from different aphid species bound *in vitro* with different affinities to *Luteoviridae*.

In our experiments, the capacity of viruliferous aphids to transmit the virus to test plants decreased with time but generally did not disappear completely. Thus, aphids could retain BYDV-PAV for many days when placed on uninfected plants (more than 3 weeks for Rp-S). These observations indicate that, under field conditions, aphids feeding on infected plants can carry

the virus for their entire life span. Virus contents in aphids as detected by ELISA can be related to transmission efficiency during the early period of transmission but are not related in the later period of daily serial transfers. The transmission efficiency may have been associated also to the retention and to natural losses of virus content in aphids during the feeding process. Our findings confirmed the results of Guo et al. (1997b) who found that the poor transmitter clones have a poor capacity to retain the virus. We believe that this difference does not imply a lower sensitivity of ELISA for BYDV at the later times, but that the concentration of virus in aphids is simply too low to be detected. Thus ELISA cannot be used as the only tool in the epidemiological studies to evaluate the presence of viruliferous vectors in the field. We also frequently observed wide variations in the amount of virus in aphids for the same time points during successive transfers. This result corroborates with previous works of Tamada and Harisson (1981) on *M. persicae* and Fargette et al. (1982) on *Acyrthosiphon pisum*.

The sub-populations assayed here are from widely dispersed locations, and the results obtained suggests that these vectors may have moved around the country. Such a pattern resembles that as seen for *M. persicae*, a vector of PLRV and of potyviruses (Van den Heuvel et al., 1993; Bourdin et al., 1998). The epidemiological advantage of the intraspecific variation phenomena could be great when allowing the virus to establish wider host and vector ranges and consequently to have better chance for survival. The recognition and understanding of the factors involved in the host–virus–vector interaction may lead to efficient control of those PAV isolates which provoke severe disease and aid in the breeding for resistant cultivars.

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