European Journal of Plant Pathology **103:** 515–519, 1997.

Variation in transmission of two BYDV-MAV isolates by multiple clones of *Rhopalosiphum padi* L.

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Accepted 25 March 1997

Key words: luteovirus, aphid clones, acquisition, inoculation, life cycle, Europe, North America

Abstract

Vector efficiency of up to 17 *Rhopalosiphum padi* L. clones originating from Europe, North America and North Africa, was evaluated by transmitting two isolates of the serotype MAV for which this species is normally an inefficient vector. When test plants were inoculated by batches of 3 aphids, both isolates were rather well transmitted by one clone (Rp5), isolate MAV2 was poorly transmitted by all other clones tested and isolate MAV11 was not transmitted by eight clones and poorly transmitted by two clones. When eight aphids were used by test plants, all clones transmitted both isolates. The epidemiological consequences of MAV transmission by some *R. padi* clones are discussed, as well as the interest of these clones for studying aphid-derived components of luteovirus transmission.

Abbreviations: BYDV – barley yellow dwarf virus; MAV – *Macrosiphum avenae* virus; PAV – *padi-avenae* virus; RPV – *Rhopalosiphum padi* virus; RMV – *Rhopalosiphum maidis* virus; SGV – *Schizaphis graminum* virus.

Barley yellow dwarf is an economically important disease of cereals worldwide. Its causal agents are a complex of luteoviruses transmitted persistently by aphids to Poaceae (Martin and d'Arcy, 1995). In Europe three viruses have been reported as widespread: RPV is transmitted most efficiently by the aphid species *Rhopalosiphum padi* L., PAV is transmitted efficiently by *R. padi*, *Sitobion avenae* F. and *Metopolophium dirhodum* (Walker) and MAV is transmitted most efficiently by *S. avenae* and *M. dirhodum* (Plumb, 1983, 1990). Degrees of vector specificity are at least partly induced by recognition between viral capsid and membrane targets in the aphid hindgut and salivary glands (Gildow, 1993; Gildow and Gray, 1993). This vector specificity is not absolute for several causes: (i) heterologous encapsidation may enable a given aphid species to transmit a virus of which it is normally not a vector (Rochow and Pang, 1961; Rochow, 1975); (ii) some isolates were reported to be transmitted by aphid species which are usually non vectors, as is the case for an Australian MAV serotype transmissible by *R. padi* (Lister and Sward, 1988), and for a Californian RPV serotype transmissible by both *S. avenae* and *Schizaphis graminum* (Rondani), and as by *R. padi* as well (Creamer and Falk, 1989); (iii) within a given aphid species, clones could differ in their abilities to transmit viruses, as shown for both stylet-borne and circulative viruses (Sadeghi et al., 1997 for review).

In the USA, Rochow (1960) reported strains of the greenbug *S. graminum* differing in their ability to transmit a BYDV-SGV isolate and four biotypes of the corn leaf aphid *Rhopalosiphum maidis* (Fitch) were shown to differ in their ability to transmit the AG-1 strain of BYDV (Saksena et al., 1964). Rochow and Eastop (1966) reported variations in transmission abilities of two morphologically different clones of *R. padi* for RMV virus. More recently Guo (1995) and Guo et al. (1996), pointed out differences in PAV, MAV and RPV transmission achievement by different clones of *R. padi* and *M. dirhodum* collected in France, and Leclercq-Le Quillec et al. (1995) noticed that some *R.*

padi alates caught alive in suction traps could transmit MAV alone.

These previous works involved only a small number of clones. The aim of the present study is to assess more widely transmission variations of two MAV isolates among a large sample of *R. padi* clones collected in different parts of the world and differing by some biological traits, in order (i) to investigate the epidemiological role of MAV transmission by this species, (ii) to find contrasting variants for further investigations on the relationships between luteoviruses and putative virus receptors in their vectors.

Two BYDV-MAV isolates were used for the transmission experiments. MAV2 was collected in le Rheu from barley in 1989 (Leclercq-Le Quillec, 1992) and causes severe yellowing to barley (cv. Express) plants. MAV11 was collected in Gembloux (Belgium) from barley and kindly supplied by H. Lapierre (INRA, Station de Pathologie Végétale, Versailles, France). It causes mild yellowing to barley (cv. Express). The aphid species used was *R. padi*, the bird cherry-oat aphid. Seventeen aphid clones were chosen and reared as described in Sadeghi et al. (1997). The biological cycle of most of them was characterized: when reared under short photophase (L: 10/D: 14) and low temperature (13 °C), holocyclic clones produce males and oviparae, androcyclic ones produce only males and parthenogenetic morphs, and anholocyclic clones are unable to produce any sexual morph (Simon et al., 1991). No sexual morphs were produced during the following experiments that were done in a growth chamber at 20+/–1°C, L:16/D:8 with a light intensity of 100,000 lx at 1 m from the source. MAV2 and MAV11 inoculum was obtained by inoculating per isolate each of twenty barley seedlings cv. Express, 2-leaf old, by 3 three fourth-instar nymphs of *S. avenae* collected on virus source-plants, and then proceeding as described in Sadeghi et al. (1997). Each plant was tested in TAS-ELISA for optical density (O.D.) comparisons, as described for BYDV by Torrance et al. (1986), Pead and Torrance (1988) and Leclercq-Le Quillec et al. (1995). For each isolate, the ten barley plants with the most similar O.D. values were kept and used as inoculum in the transmission experiments.

In a first experiment, ninety third or fourth apterous instar nymphs of each clone were allowed a 48 h acquisition access period (AAP) on source plants (MAV 2 or MAV 11). Aphids were then transferred with a fine brush to 30 barley test plants (1-leaf stage), by groups of 3 per plant and the plants were then individually caged with cellophane bags. At the end of inoculation

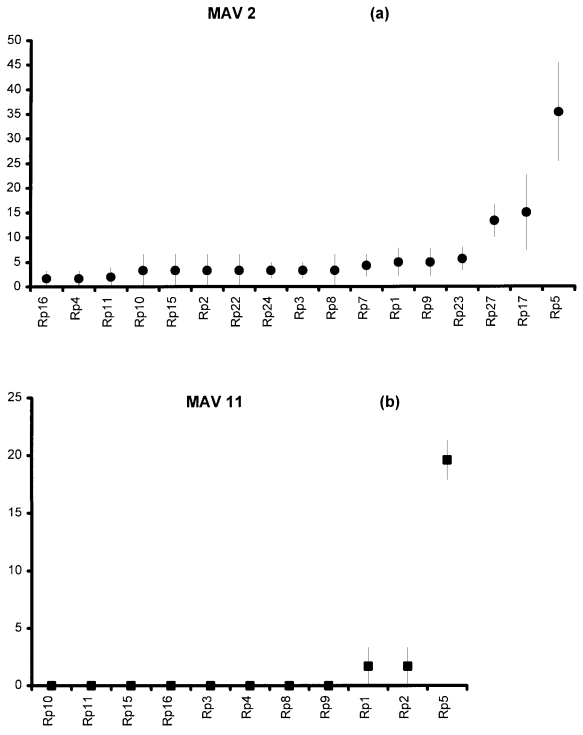

Figure 1. Mean percentage of transmission (3 repetitions of 30 plants, 3 aphids/plant) of MAV 2 (a) and MAV 11 (b) by the different clones of *R. padi*. Bars are standard errors.

access period (IAP) (5 days), aphids were killed by deltamethrin and test plants were grown in the growth chamber for 15 days before testing. The experiment was repeated three times for MAV 2 and for MAV 11 at different periods of the year. All plants were individually tested in TAS-ELISA to assess percentages of infection for each clone and isolate.

All 17 clones tested transmitted MAV2 (Figure 1), but at a very low percentage (<5%) for most of them. Clones Rp17 and Rp27 transmitted respectively at 15+/–7% and 13.3+/–3% and clone Rp5 transmitted at 35.4+/–9%. Of the 11 clones tested for MAV11 transmission, 8 did not transmit at all, 2 clones transmitted very poorly (<2%) and Rp5 transmitted at 19.6+/–2%.

A two-way variance analysis (GLM procedure type III of the SAS package) of percentages of transmission transformed in Arcsin $\sqrt{x}$ showed a significant effect of the clone (df = 10, MS = 0.55711, F = 8.51, P = 0.0001) and of the isolate (df = 1, MS = 0.80918, F = 12.36, P = 0.001). Subsequent Duncan's range tests showed: (i) that MAV2 (6.75%+/–1.9) is better transmitted than MAV11 (2.08%+/–1.9) and (ii) that Rp5 transmits at a significantly higher level than the other clones tested.

Differences among other clones were not significant (P = 0.9484). There was no interaction between both factors (df = 10, MS = 0.01348, F = 0.21, P = 0.9947), showing that the ranking of the different *R. padi* clones for MAV transmission is independant of the isolate tested.

European clones transmitted significantly better than north-american ones (P = 0.005), and holocyclic clones transmitted significantly better than the others (P = 0.0043), but there was a strong interaction between both factors, due to the fact that most holocyclic clones originated from Europe (Sadeghi et al., 1997).

Another set of experiments involved only 5 clones, Rp1, Rp3, Rp5, Rp8, Rp16. Transmission tests were done as above with groups of 3, 5 and 8 aphids per test plant. This experiment was also repeated three times.

The effect on subsequent infection rate of the number of aphids deposited for IAP on individual barley test plants was assessed by a three-way analysis of variance involving 5 clones, 2 isolates and 3 aphid groupings. Effects of clone and isolate were significant as above, but also the effect of the number of aphids (df = 2, MS = 1.69475, F = 16.34, P = 0.0003), with no interaction between the different factors. Duncan's multiple range test showed a significant difference between percentages of transmission obtained with groups of 8 aphids and those obtained with other aphid groups (3 and 5). When groups of 8 *R. padi* reared on MAV2 or MAV11 inoculum were allowed to feed on healthy plants, all clones transmitted both isolates and transmission percentages with Rp5 reached 25% and 58.4% for MAV11 and MAV2 respectively (Table 1).

There is only few reports of intraspecific variations in virus transmission by aphids and most of them compare a very small number of clones. Those on BYD-viruses mainly concern quantitative variations in transmission percentages of a given serotype by its main vector (Rochow, 1960; Saksena et al., 1964; Guo et al., 1996; Sadeghi et al., 1997). Only Rochow and Eastop (1966) and more recently Guo (1995) reported transmission studies on serotypes for which aphid species tested are usually inefficient vector, i.e variations in transmission abilities of respectively (i) 2 *R. padi* clones for RMV, normally transmitted by *R. maidis* and (ii) 2 clones of *R. padi* for MAV transmission. We compared for the first time MAV transmission by numerous *R. padi* clones collected from diverse geographical areas: our results show (i) that one MAV isolate (MAV 2) can be transmitted at least poorly by all *R. padi* clones tested, whatever their geographical origin and other biological characteristics (such as life cycle or host plant), and (ii) that there are overall differences among isolates for transmission, as MAV 11 was not transmitted by most of the clones in the same conditions, unless the number of inoculating aphid per plant was increased. Among the clones tested, one (Rp5) transmitted both isolates at a rather high level and can be considered as a genetic variant for MAV transmission. That Rp5 transmitted a PAV isolate at the same percentage than other clones tested (Sadeghi et al., 1997) seems to indicate that differences in feeding behaviour are not involved. Preliminary experiments (Sadeghi, unpublished) showed (i) that MAV2 concentrations in aphids of poor as well as good transmitting clones are similar after 5 days of acquisition-inoculation period, and (ii) that Rp5 inoculated MAV2 to test plants more rapidly (after 1-day IAP) than other clones did. This is consistent with Guo's results (1995): vector specificity in this case is not caused by either the failure to acquire the virus, or by inability of aphids to retain it, but by the capacity to inoculate it.

These results fit well with some field records and MAV transmission by *R. padi* has probably some epidemiological consequences. In France, MAV contaminations of barley fields are commonly observed in autumn, although the normal vectors of this virus (*S. avenae* and *M. dirhodum*) are absent or very scarce (Leclercq-Le Quillec, 1992). Most of the plants are infected by MAV and PAV, indicating that heteroencapsidations could play a role in MAV transmission by *R. padi*. Nevertheless, some plants are infected by MAV alone and moreover, some *R. padi* alates caught alive in suction traps could also transmit MAV alone to test plants (Leclercq-Le Quillec et al., 1995). This is a direct proof that MAV transmission by *R. padi* can occur in field conditions. That all *R. padi* clones tested transmitted at least one MAV isolate in the laboratory, and that one clone of 17 was a rather efficient vector, means that outdoor MAV transmissions by *R. padi* could be frequent and epidemiologically important, at least when *R. padi* populations contains many Rp5-type individuals. The BYDV pathosystem includes several related viruses and aphid vector species, so that it is especially adapted to studies on luteoviruses-vectors relationships. The vectors are particularly well-known concerning their biology and their molecular traits (Simon et al., 1991, 1996). Moreover, crossing experiments were achieved on some of them (Puterka and Peters, 1989; Simon, 1991) and study of the heritability of some characters becomes possible. As the vector specificity among BYD viruses has been demonstrated as the result of interactions between the

Table 1. Mean (S.E) percentage of infection obtained with 8 aphids deposited per plant for MAV2 and MAV11 inoculation. Numbers with the same letter in line are not statistically different (Duncan's range test)

	Rp1	Rp3	Rp5	Rp8	Rp16
MAV2	11.7 (6.0)b	15.5 (2.9)b	58.4 (11.3)a	18.5 (7.1)b	16.7 (5.8)b
MAV11	8.8 (3.1)b	10.0 (7.6)b	25.00 (2.89)a	6.7 (1.7)b	10.7 (0.7)b

structure protein of virus particles and the different targets of the membrane system of the aphid (Gildow, 1993; Gildow and Gray, 1993; Wang et al., 1995), the disposal of genetically close variants for transmission, especially recombinants issued from self crosses of Rp5, can provide useful material for studying aphid derived components involved in luteovirus transmission.

Acknowledgements

Authors are especially grateful to Dr J.C.Simon (INRA, Laboratoire de Zoologie, Le Rheu, France) for supplying the *R. padi* clones, to H. Lapierre (INRA, Station de Pathologie Végétale, Versailles, France) for the gift of polyclonal antibodies and of the MAV 11 isolate, and for usefull discussions, and to Y. Robert (INRA, Laboratoire de Zoologie, Le Rheu, France) for english revision of the manuscript.

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