European Journal of Plant Pathology **107:** 167–173, 2001.

The effect of temperature on variation in transmission of a BYDV PAV-like isolate by clones of *Rhopalosiphum padi* and *Sitobion avenae*

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Accepted 9 October 2000

Key words: aphid clones, Greece, life cycle, luteovirus, virus inoculation

Abstract

The efficiencies of seven clones of *Rhopalosiphum padi* and five clones of *Sitobion avenae* (originating from Greece and the United Kingdom) as vectors of barley yellow dwarf virus (PAV-like isolate) were evaluated at 5, 10 and 15 °C. When inoculation took place at 5 or 10 °C, clones of *R. padi* differed in their ability to transmit. At 15 °C there were no differences in the vectoring ability of different clones. For *S. avenae*, there were no interclonal differences in the transmission efficiency at any of the temperatures. The epidemiological consequences of differences in virus transmission at different temperatures are discussed.

Abbreviations: BYDV – barley yellow dwarf viruses; CYDV – cereal yellow dwarf viruses; MAV – BYDV–MAV species in the genus *Luteovirus*, family *Luteoviridae*, transmitted by *S. avenae*; PAV – BYDV–PAV species in the genus *Luteovirus*, family *Luteoviridae*, transmitted by *R. padi* and *S. avenae*; RMV – BYDV–RMV species unassigned within the family *Luteoviridae*, transmitted by *R. maidis*; RPV – CYDV–RPV species in the genus *Polerovirus*, family *Luteoviridae*, transmitted by *R. padi*; SGV – BYDV–SGV species unassigned within the family *Luteoviridae*, transmitted by *Schizaphis graminum*.

Introduction

BYDV and CYDV are economically important diseases of cereals worldwide. The causal agent is a complex of viruses belonging to the family *Luteoviridae* (Mayo and D'Arcy, 1999) which are transmitted in a persistent manner (Ossiannilsson, 1966) by more than 25 species of aphids (Blackman et al., 1990). The known host range of the viruses includes more than 150 species of the family Poaceae (Gould and Shaw, 1983).

Several factors influence the efficiency and specificity of transmission of BYDV and CYDV. Factors associated purely with the aphid vectors include developmental stage, morph and clone. Factors associated with the virus include virus source (plant species, and age), mixing of virus components in infections with multiple BYDV or CYDV isolates (transcapsidation), inoculation access period and acquisition access period. Temperature plays the most important environmental role in variation in transmission efficiency (Creamer and Falk, 1990; Gill, 1969; Gray et al., 1991; Hu et al., 1988; Lowles et al., 1996; Power and Gray, 1995; Rochow, 1969; 1982). Studies on variation in luteovirus transmission by aphids have shown that transmission depends more on the interaction between aphid clones and virus isolates than on the properties of solely the virus or the vectors (Bourdin et al., 1998). Aphid clones have been shown to differ in molecular characteristics (Carvalho et al., 1991; Black et al., 1992; Simon et al., 1996) and/or biological traits, such as ability to produce sexual morphs (Blackman, 1971; Simon et al., 1991), host-plant preference (Bournoville, 1971;

Via, 1991; Caillaud et al., 1995) and virus transmission efficiency (Rochow, 1960; Saskena et al., 1964; Rochow and Eastop, 1966; Guo et al., 1996; Sadeghi et al., 1997a,b). Although interclonal variations in transmission efficiency of the same isolate have been demonstrated (Rochow, 1960; Saskena et al., 1964), most studies of aphid-BYDV/CYDV relationships have been focussed on variation among species. No differences in the BYDV/CYDV vectoring ability of clones of *R. padi*, *S. avenae* and other species were reported by Rochow (1958), Bruehl (1958), Smith and Richards (1963), Gill (1967) or Gildow and Rochow (1983). In contrast Rochow (1960) reported differences in the efficiency of biotypes of *S. graminum* transmitting a BYDV–SGV isolate, and Saskena et al. (1964) showed differences in the ability of four biotypes of *R. maidis* to transmit an isolate of BYDV. Rochow and Eastop (1966) reported variation in transmission abilities of two clones of *R. padi* for BYDV–RMV, and noted that differences were less pronounced when experiments were carried out at 30 °C than at lower temperatures. Guo et al. (1996), and Sadeghi et al. (1997a,b) showed differences among PAV, MAV and RPV in transmission efficiency by different clones of *R. padi*, *S. avenae* and *Metopolophium dirhodum*.

The aim of this study was to assess variation in transmission efficiency of one PAV isolate from Greece among seven clones of *R. padi* and four clones of *S. avenae* collected from different sites in Northern Greece and one clone of *S. avenae* obtained from Harpenden, Herts, UK, under three different temperatures, and to consider the epidemiological implications of the results.

Material and methods

Virus isolate

A PAV-like isolate of BYDV from Greece was used. This isolate caused very severe stunting and yellowing in barley (*Hordeum vulgare*) cv Athenaida. The isolate was maintained on wheat seedlings at 10 °C and was used between 20 and 30 days from inoculation.

Aphid species

A clone of *S. avenae* (Sa 12) was collected from IACR-Rothamsted and seven clones of *R. padi* (Rp 1–Rp 7) and four of *S. avenae* (Sa 8–Sa 11) were collected from five different sites in northern Greece during 1997 and 1998 (Table 1). The aphids were kept under controlled laboratory conditions at 18 °C and L:16/D:8 in perspex tubes (Austin et al., 1991). The leaves that aphids fed on were analysed by ELISA to ensure that the aphids were virus free. After three to four weeks the aphids were transferred to wheat under controlled laboratory conditions (18 °C, L:16/D:8). The culture was maintained at low densities on plants by regular transfer of apterae to clean host plants. The biological cycle of all aphids was characterised when reared under short photophase (L:8/D:16) at 13 ± 0.5 °C (Smyrnioudis, 2000). Holocyclic clones produce males and oviparae; androcyclic clones produce only males and parthenogenetic morphs and anholocyclic clones are unable to produce any sexual morphs. All the clones showed an androcyclic response except one clone of *R. padi* that showed an anholocyclic response and the clone of *S. avenae* from the UK that showed a holocyclic response (Table 1).

Table 1. Origins of aphid clones used in the experiment

Species	Clone	Collection site	Response	Date of collection	Host plant
R. padi	Rp 1	Ptolemaida	Anholocyclic	10/08/1997	Maize
R. padi	Rp 2	Florina	Androcyclic	10/08/1997	Maize
R. padi	Rp 3	Florina	Androcyclic	30/04/1998	Maize
R. padi	Rp 4	Serres	Androcyclic	10/08/1997	Maize
R. padi	Rp 5	Serbia	Androcyclic	30/04/1998	Wheat
R. padi	Rp 6	Ptolemaida	Androcyclic	10/08/1997	Maize
R. padi	Rp 7	Kozani	Androcyclic	06/02/1997	Wheat
S. avenae	Sa 8	Serbia	Androcyclic	10/08/1997	Maize
S. avenae	Sa 9	Serbia	Androcyclic	30/04/1998	Wheat
S. avenae	Sa 10	Serres	Androcyclic	30/04/1998	Wheat
S. avenae	Sa 11	Thessaloniki	Androcyclic	10/08/1997	Maize
S. avenae	Sa 12	UK	Holocyclic	1992	Wheat

Experimental plants

Durum wheat seedlings (cv Sifnos) were planted in Jiffy® seed trays in 50% coarse sand and 50% peat enriched with slow release fertiliser (Osmocote®). The plants were watered from below by placing in a water-filled tray.

Virus inoculation of source plants

Twelve seedlings for each clone at each temperature were set up as source plants. Each seedling was inoculated at the two-leaf stage by three to five fourth instar apterous *R. padi* taken from virus source plants. The aphids were placed inside clip cages (Awmack, 1997) and left for 3 days at 18 °C L:16/D:8. After that period all seedlings were sprayed with the systemic insecticide Pirimor® (ICI, 50% w/w pirimicarb as a water dispersible granule, dilution rate 0.5 g/l) and kept at 15 °C for 15 days until tested by TAS-ELISA.

Virus acquisition

Virus acquisition was done in a controlled environment cabinet at 23 °C and L:16/D:8. One hundred fourth instar apterae of each clone were transferred to the source plants (one clone per plant) for a three day acquisition access period. Adult apterae were removed after 72 h and kept in glass tubes, one for each clone, without plant material, for an hour before use in the experiment. Plants were inoculated in growth chambers at 5, 10 or 15 °C. For each clone in each chamber twelve apterous adults from the glass tubes (see above) were transferred singly with a fine paint brush to twelve separate test plants (Growth Stage 12; Zadoks et al., 1974) and were confined by use of clip cages (Awmack, 1997). There were therefore 144 plants in each chamber in total. Aphids were allowed to feed for 72 h and then were identified as alive or dead and living aphids killed using Pirimor®. The plants were transferred to a glasshouse and checked by TAS-ELISA after 20 days. The entire experiment from source plant inoculation was repeated six times. On each occasion the temperatures were randomised to growth chambers according to Latin square design.

Enzyme-Linked Immunosorbent Assay (ELISA)

Each wheat plant was tested for the PAV isolate. The method used was based on that given by Clark and Adams (1977) and described in more detail for BYDV by Torrance et al. (1986). Polyclonal antibody (Adgen Diagnostics, Auchincruive, Ayr, Scotland, UK) was used for coating, while monoclonal detection (probe) antibody (Adgen Diagnostics) was used after addition of the sample. Test samples were assayed together with samples known to be virus free and with samples known to be infected. Samples with Optical Density values greater than three times that given by the healthy sample were considered infected.

Statistical analyses

The transmission efficiencies (percentage plants infected) of the PAV isolate by different *R. padi* and *S. avenae* clones were analysed separately using analysis of variance (Genstat 5 Committee, 1997). F-statistics with degrees of freedom and probability values are given in parentheses. The few plants on which dead aphids were found were excluded from the analyses.

Results

The transmission efficiency of the PAV isolate differed between *R. padi* clones ($F_{6,90} = 5.14$, $P < 0.001$) and between temperatures ($F_{2,10} = 57.81$, $P < 0.001$). There was only slight evidence of an interaction between clones and temperature ($F_{12,90} = 1.71$, $P = 0.08$). The assessment of vectoring efficiency of different *S. avenae* clones suggested that there was no difference between them ($F_{4,60} = 1.49$, $P = 0.215$) but there was a significant difference in vectoring efficiency between temperatures ($F_{2,8} = 12.30$, $P < 0.01$) and there was also a significant interaction ($F_{8,60} = 3.17$, $P < 0.01$) (Figure 1). For *R. padi* at 5 °C, significant differences were found between the most efficient clones, Rp 4 and Rp 5, and the least efficient clones, Rp 6 and Rp 7. Clones Rp 2 and Rp 3 were significantly different from Rp 6 but not from Rp 7. The most efficient clone of *R. padi*, Rp 4, transmitted the PAV isolate to 61% of tested plants at 5 °C, but only 39% transmission was obtained at that temperature by the least efficient vector, Rp 6. Among the clones of *S. avenae* at 5 °C there were no significant differences. The most efficient clone, Sa 10, transmitted the PAV isolate to 13% of tested plants, but only 6% transmission was obtained by the least efficient clones, Sa 11 and Sa 12.

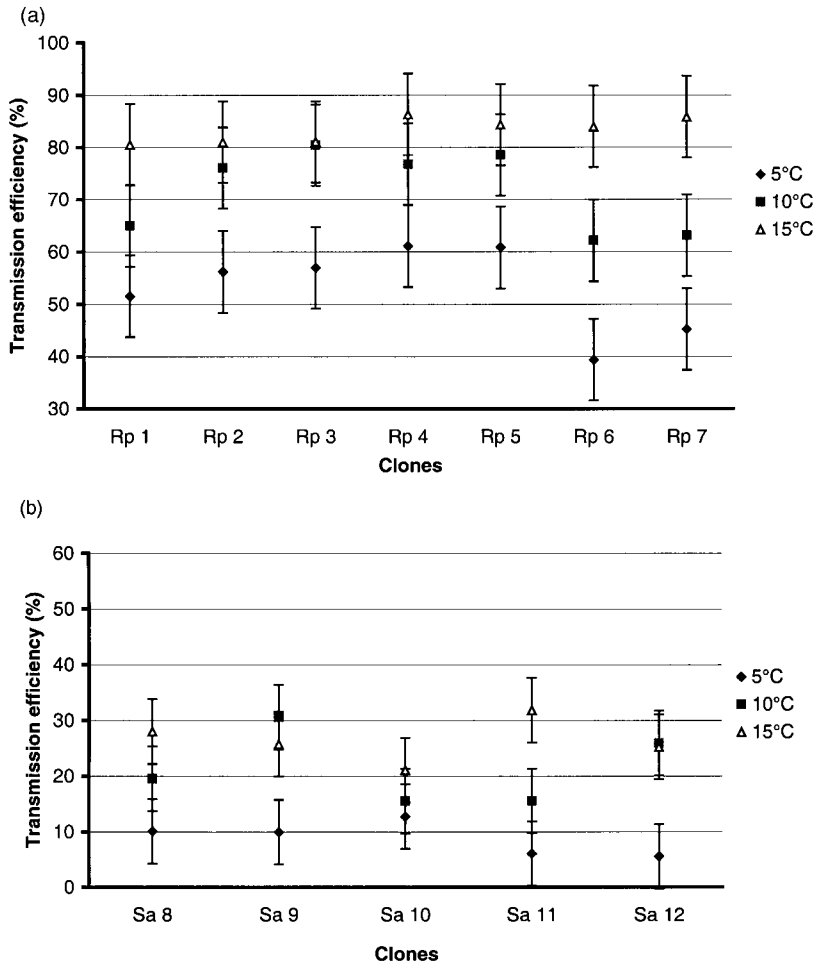

Figure 1. Mean percentages ($n = 6$) of PAV transmission to wheat plants by different clones of (a) *R. padi* and (b) *S. avenae* at three temperatures. Bars represent 95% confidence intervals about the means.

At 10 °C the same pattern was followed by the *R. padi* clones as at 5 °C with significant differences between the most efficient clones, Rp 3, Rp 4 and Rp 5, and the least efficient clones, Rp 6 and Rp 7. The most efficient clone of *R. padi* at 10 °C, Rp 3, transmitted the virus isolate to 80% and the least efficient, Rp 6, to 62% of plants. For the *S. avenae* clones there was a significant difference between the most efficient clone, Sa 9, and the least efficient clones, Sa 10 and Sa 11. The transmission efficiency for the clone Sa 9 was 30% but only 15% for the least efficient clones.

At 15 °C the pattern followed by *R. padi* at 5 and 10 °C changed. The seven clones of *R. padi* transmitted the virus to between 80% (Rp 1) and 86% (Rp 4) of plants but this difference was not statistically significant. For *S. avenae* the most efficient clone, Sa 11, transmitted the virus to 32% of plants and the least efficient, Sa 10, to 21% but this difference was again not statistically significant.

For *R. padi* almost the same pattern was followed at 10 and 5 °C, but not at 15 °C, with respect to the relative efficiencies of different clones. However, for *S. avenae* the most and least efficient clone changed between temperatures. Temperature had an effect on vectoring efficiency for both species with significantly greater transmission at the higher temperature. The mean transmission efficiencies for *R. padi* at 5, 10 and 15 °C were 53.0%, 71.7% and 83.3%, respectively. For *S. avenae* the mean transmission efficiencies at 5, 10 and 15 °C were 8.9%, 21.4% and 26.4%, respectively.

Discussion

The purpose of this investigation was to compare the ability of a range of clones from two aphid species to transmit the BYDV PAV-like isolate from Greece at different temperatures. Although all the aphid clones acquired and retained the virus, a substantial variation in vectoring ability between clones of *R. padi* was observed. The transmission efficiency of *R. padi* ranged from 39% to 86%. Further investigation is needed to demonstrate the reason for this variability. It could be associated with barriers to circulation of the virus within the aphid or to poor capacity for virus retention by the aphids due to aphid–virus or aphid–host plant interactions (Gildow and Rochow, 1980; Gildow, 1987). All these factors contribute to the overall transmission process and if they are subject to genetic heterogeneity within aphid species this may explain interclonal variation in transmission efficiency.

The results of the transmission studies demonstrated that temperature plays a significant role in variability of vectoring efficiency among clones of *R. padi*. The differences found at 5 °C and 10 °C were not shown at 15 °C. Temperature could theoretically influence virus content in plants, virus stability within vector aphids and the feeding pattern of aphids. In this trial the explanation is most likely to be the alteration of the virus–aphid interaction and/or the aphid–host plant interaction. At low temperatures the feeding rate of an aphid decreases. It is possible that the efficiency of barriers to circulation of the virus then becomes more important and may play a more significant role in variability of vectoring ability than at higher temperatures where transmission is more efficient. It has been suggested that high temperature alters the virus–vector interaction (Rochow and Eastop, 1966). Possible explanations include alteration of virus structure to expose critical transmission domains on the virus particle or a change in the physiological barriers within the aphid allowing the virus to interact with and be transported by mechanisms not normally available at lower temperatures (Gildow, 1999). Generally, differences in transmission among clones of an aphid species have been found in adverse conditions, such as poor transmissibility of an isolate (Bourdin et al., 1998), inefficient vector capacity (Sadeghi et al., 1997b), short inoculation and acquisition access periods (Guo et al., 1996), or low temperatures.

For *S. avenae*, there were overall differences in transmission at different temperatures, with higher transmission at 15 °C than at 10 or 5 °C, but there were no overall differences between clones.

The aphid clones used were collected from five different sites in northern Greece in an area of about 400 km^2. Because the aphid clones were not genotyped, it is possible that some which had similar responses were genetically similar. However, the aim of this work was to assess the effect of temperature on virus transmission by aphid clones representative of northern Greece and so knowledge of the genetic substructure, whilst helpful in explaining the degree of difference in transmission efficiency, is subordinate to the need for random samples.

R. padi is generally an efficient vector of BYDV–PAV isolates. In our experiment, even at 5 °C with the least efficient clone, the transmission efficiency was 39%. The most efficient vector at the same temperature transmitted the isolate used to 61% of the plants tested. That 22% difference in transmission may play an important role in BYDV epidemiology during the winter when the temperature can be that low. Seasonal prevalence of efficient or inefficient vector clones in a given area could greatly affect the course of an epidemic, especially when adverse conditions occur. When differences in vectoring efficiency of aphid clones are assessed, great care should be taken when factors affecting the physiological activity of aphids and the interaction between virus-vectors are not optimal. Effects of these factors, like temperature in our trial, could significantly affect the efficiency of the vectors.

Acknowledgements

Ilias Smyrnioudis is sponsored by the EU Research Training Grant Contract FAIR-BM-960947. IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom.

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