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Research Note

Pseudococcus affinis Mask., new vector of grapevine trichoviruses A and B

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S u m m a r y : Grapevine trichovirus A (GVA) and grapevine trichovirus B (GVB) were successfully transferred with bulk transmission trials under controlled conditions, from infected grapevines to herbaceous hosts by *Pseudococcus affinis* Mask., a pseudococcid mealybug that may attack grapevines. *P. affinis* is the fourth mealybug species capable of vectoring GVA and GVB, confirming that transmission by mealybugs of grapevine trichoviruses may not be species-specific.

K e y w o r d s : trichoviruses, transmission, mealybugs, epidemiology.

Introduction: *Pseudococcus affinis* Mask. is a polyphagous pseudococcid mealybug present in several areas of the world. In the southern emisphere (McLachlan 1970) and Western America (Wilkey and McKenzie 1961; Gonzales 1983; Ripa and Rojas 1990), it is also reported as a grapevine pest. In Sardinia (Southern Italy) *P. affinis* thrives primarily on the sclerophyllous shrubs of the Mediterranean scrub and, although it apparently does not attack *Vitis* species in the field, it can occasionally be found colonizing glasshouse-grown vines. Thus, it may represent a potential virus vector, similarly to other mealybugs of the *Planococcus* and *Pseudococcus* genera (reviewed by Bovey and Martelli 1992; Martelli 1993).

This paper reports the results of trials in which the vectoring ability of *P. affinis* was experimentally tested.

Materials and methods: A *P. affinis* population was collected from a naturally infested myrtle plant (*Myrtus communis*) near Sassari and transferred to young sprouts of virus-free potatoes growing in glass jars in a climatized greenhouse at a temperature ⊕ 25 °C. Under these conditions, the mealybugs adapted readily to the new host producing a new generation in about 5 weeks.

Mealybug populations thriving on potatoes were used for bulk transmission tests made in a growth chamber at 25 °C and 16 h photoperiod (3,500 lx). Infected grapevine rootlings served as donor and herbaceous plants as recipient hosts.

Two sets of trials were carried out:

T r i a l 1 : Donors were pot-grown rooted cuttings from a cv. Italia vine (accession I 9/17) that had indexed positive for corky bark and was known to be infected by a necrotic strain of grapevine trichovirus B (GVB) (Garau *et al.* 1993; Boscia *et al.* 1994) and ordinary isolates of grapevine trichovirus A (GVA) and grapevine leafroll-associated virus 3 (GLRaV-3). Fragments of *P. affinis*-infested potato sprouts were placed on grapevine rootlings and the mealybugs were let to multiply undisturbed for 40 d. Then, no less than 30 1st and 2nd instar crawlers were removed from grapevine leaves with the piece of tissue on which they were feeding and were placed on each of 5 plants of *Nicotiana occidentalis, N. cavicola* and *Gomphrena globosa.* Controls consisted of an equal number of plants of the same species that were infested with mealybugs reared on healthy potatoes.

T r i a l 2 : Donors were rootlings from a cv. Italia vine (accession I 7/8) infected by GVA, GLRaV-3, grapevine corky bark-associated closterovirus (GCBaV) and grapevine fanleaf nepovirus (GFLV). Mealybugs were placed on donor vines and after an acquisition period of 30 d were transferred with the same technique as above to 3 plants each of *N. occidentalis, N. clevelandii,* and *N. benthamiana* Plants of the same species exposed to non viruliferous mealybugs served as controls.

All herbaceous hosts were observed for symptom expression and were individually checked in ELISA (Clark and Adams 1977) and immunoelectron microscopy (IEM) (Milne and Luisoni 1977) for virus detection and identification.

Results and discussion: In both trials *P. affinis* thrived and multiplied very well on grapevine rootlings. It also adapted to the different herbaceous hosts, although with some difficulty. However, many of the mealybug crawlers transferred from grapevines fed and survived on these plants.

In the first trial, a light vein clearing began to show on a *N. occidentalis* plant after 10 d from exposure to mealybugs that had fed on donor vines. In the week that followed, the symptoms became stronger and indistinguishable from those induced by necrotic isolates of GVB following mechanical inoculation (Garau *et al.* 1993; Boscia *et al.* 1994) and developed on the remaining 4 *N. occidentalis* and the 5 plants of *N. cavicola.* On this latter host the symptomatology was the same as that induced by mechanically inoculated GVB isolates (Garau *et al.* 1993; Boscia *et al.* 1994). All symptomatic *Nicotiana* plants were ELISA and IEM positive for GVB but did not react with antisera to GVA and GLRaV-3. Controls were always negative.

All 5 *G. globosa* seedlings that had been exposed to presumably viruliferous mealybugs reacted in 10-15 d with a systemic interveinal mottling of varying intensity, whilst the controls remained symptomless. Symptomatic plants were ELISA and IEM positive for GVA, but not for GVB and GLRaV-3. The presence of GVA was further ascertained by immunogold labelling IEM (F. Faoro, personal communication) and through subinoculations to *N. benthamiana.* None of the 3 viruses was detected in the controls.

In the second trial, 2 of 3 *N. benthamiana* seedlings exposed to presumably viruliferous mealybugs, showed in

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about 10 d symptoms indistinguishable from those induced by GVA following mechanical inoculation. The remaining seedling and those of *N. occidentalis* and *N. clevelandii* remained symptomless, similarly to the controls. Symptomatic plants reacted positively for GVA in ELISA and IEM but were negative for the other viruses tested (GLRaV-3, GCBaV, GFLV). All asymptomatic plants and the controls were ELISA negative.

The results of the present trials provide evidence that *P. affinis* is capable of acquiring GVA and GVB from diseased vines and of moving it to herbaceous hosts with bulk transmission experiments. Actually, it looked as if that this mealybug species could discriminate between GVA and GVB by picking up the 2 viruses from a mixture and transmitting them individually to different herbaceous hosts. This may be due either to a really differential vectoring ability of *P. affinis* and/or to the diverse susceptibility of the herbaceous indicators to these viruses. In fact, GVA, but not GVB, can infect *G. globosa* (BOSCIA *et al.* 1994; MARTELLI *et al.* 1994), whereas GVB seems to be more readily transmissible and aggressive than GVA to *N. occidentalis* and *N. cavicola.*

Whatever the explanation, the point remains that *P. affinis* is a vector of grapevine trichoviruses, thus joining the list of the hitherto identified mealybug vector species (BOVEY and MARTELLI 1992; MARTELLI 1993), and adding strength to the notion that mealybug transmission of these viruses may not be species-specific.

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