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Broad bean mottle virus in Morocco; curculionid vectors, and natural occurrence in food legumes other than faba bean (Vicia faba)

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Abstract

Broad bean mottle virus (BBMV) was transmitted from infected to healthy faba-bean plants by the curculionid weevils *Apion radiolus* Kirby, *Hypera variabilis* Herbst, *Pachytychius strumarius* Gyll, *Smicronyx cyaneus* Gyll, and *Sitona lineatus* L. The latter appeared to be an efficient vector: acquisition and inoculation occurred at the first bite, the rate of transmission was c. 41%, and virus retention lasted for at least seven days. *S. lineatus* transmitted the virus from faba bean to lentil and pea, but not to the three genotypes of chickpea tested. This is the first report on the genera *Hypera*, *Pachytychius*, and *Smicronyx* as virus vectors, and on *A. radiolus*, *H. variabilis*, *P. strumarius*, and *S. cyaneus* as vectors of BBMV.

Out of 351 samples of food legumes with symptoms suggestive of virus infection, 16, 11, 19, and 17% of the samples of chickpea, lentil, pea, and common bean, respectively, were found infected when tested for BBMV in DAS-ELISA. This is the first report on the natural occurrence of BBMV in chickpea, lentil, pea, and common bean. The virus should be regarded as a food-legume virus rather than a faba-bean virus solely, and is considered an actual threat to food legume improvement programmes.

Additional keywords: *Apion, Cicer arietinum, Curculionidae, Hypera, Lens culinaris, Pachytychius, Phaseolus vulgaris, Pisum sativum, Sitona, Smicronyx,* weevil transmission.

Introduction

Earlier surveys of faba bean (*Vicia faba* L.) for viruses in Morocco have shown broad bean mottle virus (BBMV) to be prevalent. Its incidence per field ranged from 1 to 33%, and the virus was found widespread in the faba-bean growing areas, especially in the central and northern parts of the country (Fortass and Bos, 1991). It was later reported to be seed transmitted in faba bean, chickpea, and pea at transmission rates of c. 1.2, 0.9, and 0.1%, respectively (Fortass and Bos, 1992).

The prevalence of the virus in Morocco and its high incidence in some fields suggest the existence of efficient vectors, but information on vector transmission of BBMV is still limited. Walters and Surin (1973), in the USA, where the virus has not been reported to occur naturally, were the first to report experimental transmission of BBMV by the striped cucumber beetle (*Acalymma trivittata*), the spotted cucumber beetle (*Diabrotica undecimpunctata*), and the grape colaspis (*Colaspis flavida*) at transmission rates of 9.67, 7.76, and 6.66%, respectively. Later, a similarly poor transmission (5 to 6%) by the leaf weevil *Sitona lineatus* var. *viridifrons* Motsch was reported in Portugal (Borges and Louro, 1974). Cockbain (1983) showed that BBMV is transmitted by *Apion* spp., and a transmission rate of 20% was obtained with *A. vorax*. Recently, Makkouk and Koumari (1989) reported that *A. arrogans* is a vector of BBMV in Syria.

Another ecological feature suggesting the potential importance of the virus is its wide host range among legume species (Makkouk et al., 1988). Recently, some promising breeding lines of faba bean, chickpea, pea, and lentil were tested and found susceptible and sensitive to the virus (Fortass and Bos, 1992). Symptoms, such as necrosis, produced in these species are likely to be overlooked as caused by BBMV. This suggests a wide-spread, yet unknown, occurrence of the virus in food legumes other than faba bean, and the possible existence of vectors spreading the virus between food-legume crops.

This paper reports on two important aspects of the ecology of BBMV in Morocco i.e. its vectors and natural occurrence in food legumes of importance in the country.

Materials and methods

Insect species. The curculionid weevils *Apion radiolus* Kirby, *Sitona lineatus* L., *Hypera variabilis* Herbst, *Smicronyx cyaneus* Gyll, and *Prachytychius strumarius* Gyll (Fig. 1) were collected from faba-bean fields in the areas of Meknes, Fes and Taounate in Morocco. They were maintained in the greenhouse on *V. faba* 'Aguadulce'. All the insects were collected from apparently healthy faba bean plants, and were transferred at 2- to 3-day intervals to three successive healthy 'Aguadulce' plants in order to eliminate viruses acquired by the beetles in the field. Field-collected insects were used since the larvae are soil feeders and the rearing procedure is time consuming. The weevil species were identified by A. Boughdad, Département de Zoologie Agricole, ENA, Meknès, Morocco.

Plant species. Plants of *V. faba* 'Aguadulce', mechanically inoculated with the Moroccan isolate VN5 (Fortass and Bos, 1992), were used as virus source. The same cultivar was used for assays. In case of transmission of BBMV by *S. lineatus*, other food legumes were used as assay plants and are listed in Table 1. These genotypes were provided by M. El Yamani, INRA-MIAC, Settat, Morocco.

Transmission tests. The procedure of transmission was according to Bakker (1974) as used for rice yellow mottle virus. Depending on the acquisition access period (AAP), two approaches were used. For AAPs of 12, 24, and 48 h, the complete plants were used as a source of virus, while for AAPs of ¼, ½, 1, and 10 h, detached leaves placed in Petri dishes were used as a virus source. All insects were submitted to pre-acquisition starving for 4 to 24 h. The assay plants were used at the 2- to 3-leaf stage, and the number of insects per

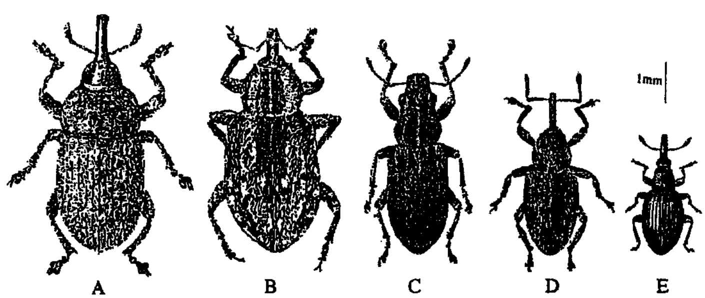

Fig. 1. Drawings and comparative average sizes of the weevil vectors of broad bean mottle virus. A: *Pachytychius strumarius*, B: *Hypera variabilis*, C: *Sitona lineatus*, D: *Smicronyx cyaneus*, and E: *Apion radiolus*. (Rearranged after Hoffmann, 1959).

plant varied from 1 to 3. All assay plants used in the transmission studies were kept in the greenhouse for 2 to 3 weeks, and then tip leaves were stored at –20 °C for later testing in ELISA.

Quantification of transmission by S. lineatus. In order to assess the acquisition and inoculation access periods, 412 individuals of *S. lineatus* were given AAPs of ¼, ½, 1, 10, 12, and 24 h and 1, 2, and 4 days of inoculation access periods (IAPs) on 190 'Aguadulce' plants, with 1 to 3 insects per plant. The retention period of BBMV by *S. lineatus* was determined by daily transfers of viruliferous insects to healthy plants, using 1 and 2 individuals per plant. Serial transfers to healthy plants following the first bite were also done with 4 insects (1 per plant). The transmission rate of BBMV by the insect was determined as the ratio of the number of infected plants to the total number of tested plants, using single insects and 24 h as AAP and IAP.

Transmission to other food legumes by S. lineatus. A total of 656 individuals of *S. lineatus*, given an AAP of 24 h on infected 'Aguadulce', were transferred to 286 plants of lentil (*Lens culinaris*), 20 plants of chickpea (*Cicer arietinum*), and 22 plants of pea (*Pisum sativum*). The IAP was 24 h and the number of insects per plant varied from 1 to 3.

ELISA testing. The assay plants used in the transmission experiments were tested for BBMV in double-antibody sandwich ELISA as by Clark and Adams (1977). The antiserum used had been raised against the Moroccan isolate MV90-85 (Makkouk et al., 1988). The g-globulins were used at 1 μg/ml and the alkaline phosphatase conjugated antibodies were diluted to 1:1000 in the extraction buffer (0.03 M potassium phosphate buffer, pH 7.7). Healthy plants not fed upon by insects were used as negative controls. The leaf material was ground in the extraction buffer 1:4 (w/v). The insects used in the serial transfers were tested for virus retention by ELISA. They were ground with pestle and mortar at a ratio 1 insect per 0.5 ml extraction buffer. The extracts were then centrifuged for 10 min at low speed (1000 g), and the supernatants were used in ELISA (200 μl per well). Non-viruliferous insects kept on virus-free plants were used as negative controls. The sample was considered positive when the absorbance value exceeded that of the negative control plus three standard deviations. The 'Aguadulce' plants infected by BBMV-VN5, and used in the transmission tests as source plants, were used as a positive control.

Survey of other food legumes and weeds for BBMV. A series of surveys were conducted at different times of the year in the central and northern parts of the country in fields of chickpea, pea, lentil, and common bean (*Phaseolus vulgaris*). Per species, 24 fields were surveyed, and a total of 351 samples of food legumes and 102 samples of legume weeds, showing symptoms suggestive of virus infection, were collected. They were tested for BBMV by ELISA as described above. The weed species were identified by A. Tangi (INRA-MIAC, Settat, Morocco).

Results

Transmission of BBMV by vectors

Sitona lineatus. The results of the experiments are shown in Table 1. The insect species transmitted BBMV from infected to healthy faba bean at all AAPs and IAPs tested. A higher transmission rate was obtained with three individuals than with single insects per plant, and an AAP of 15 min was enough for *S. lineatus* to transmit the virus.

Table 1. Transmission of broad bean mottle virus to faba bean by Sitona lineatus, at different acquisition and inoculation access periods.

Number of insects per plant	Acquisition access period (h)	Inoculation access period (days)	Transmission results (%)[a]
1	1/4	1	33 (1/3)
	1/2	1	14 (2/14)
	48	1	12 (2/16)
	1	2	5 (1/17)
	12	2	12 (2/16)
	24	2	45 (9/20)
	48	1	12 (2/16)
3	1/4	1	75 (6/8)
	10	1	72 (8/11)
	24	1	33 (4/12)
	48	1	85 (17/20)
	1	2	50 (5/10)
	24	2	50 (10/20)
	1/2	4	47 (8/17)
	12	4	47 (9/19)

[a] Proportion of infected plants out of inoculated plants given in parentheses.

Smicronyx cyaneus. *S. cyaneus* is also able to transmit BBMV from faba bean to faba bean. Transmission occurred at AAPs of 24 h, and a high rate (6/8) was obtained after a 48-h AAP and a 24-h IAP. No transmission was obtained after short AAPs of 15 or 30 min, but the number of plants used for assaying was not high. In daily transfers of two individuals per plant given an AAP of 24 h and an IAP of 48 h, it was found that the virus persisted in *S. cyaneus* for 48 h only (data not shown).

Other Curculionidae. All three species tested transmitted the virus to faba bean. Given an IAP and an AAP of 24h each, *Apion radiolus* transmitted BBMV to 2 plants out of 12, *Hypera variabilis* to 4 out of 6, and *Pachytychius strumarius* to 2 out of 10. The number of insects collected was very low, thus limiting further studies with these species.

Quantification of the transmission by S. lineatus. The shortest AAPs and IAPs tested were 15 min and 24 h respectively, they still led to virus transmission. Of a total of 100 plants of 'Aguadulce' tested for transmission of BBMV by single *S. lineatus*, given an AAP and an IAP of 24 h each, 41 plants were found to be infected. The rate of transmission was thus estimated to be 41%. The results of the daily transfers show that all the plants used in the experiment were infected, implying that *S. lineatus* retains and transmits the virus until after the seventh transfer. Moreover, in serial transfers following the first insect feeding damage, it was found that the first insect bite already led to virus transmission (data not shown).

Transmission by S. lineatus to other food legumes. The results in Table 2 show that *S. lineatus* transmits BBMV from *V. faba* to some but not all genotypes of *L. culinaris* and *P. sativum*, but not to the three genotypes of *C. arietinum* tested.

Table 2. Transmission of broad bean mottle virus from faba bean to chickpea, lentil, and pea by Sitona lineatus, given acquisition and inoculation access periods of 24 h (two insects per plant).

Species and genotype	Transmission results (%)[a]	
Chickpea		
F 84-82 C	0*	(0/11)
F 88-75 C	0	(0/13)
F 84-182 C	0	(0/6)
Lentil		
ILL 6212	0	(0/26)
L 121	7	(2/26)
L 56	19	(36/189)
ILL 6001	62	(28/45)
Pea		
SM 790031	0	(0/9)
PH 135 B	66	(4/6)
P 343	71	(5/7)

[a] Proportion of infected plants out of inoculated plants given in parentheses.

Natural occurrence of BBMV in other food legumes and legume weeds. Among the 102 symptom-bearing samples of the legume weeds *Trifolium alexandrinum*, *Medicago scutellata*, *Medicago hispida*, and *Vicia sativa*, none was found infected with BBMV when tested in ELISA. In contrast, the legume crops chickpea, pea, lentil, and common bean, were found infected with the virus. Table 3 shows that c. 16, 11, 19, and 16% of the symptom-bearing samples of chickpea, lentil, pea, and common bean, respectively, were found infected by BBMV. The virus was found in all four food legume crops in the areas surveyed, except in lentil in the area of Meknes.

Table 3. ELISA testing of symptom-bearing samples of food legumes collected from fields.

Food legumes	Area of collection		Results *
Cicer arietinum	Meknès		4/35
	Taounate		11/58
		Total	**15/93** (16%)
Lens culinaris	Meknès		0/14
	Fès		5/42
	Taounate		8/58
		Total	**13/114** (11%)
Phaseolus vulgaris	Meknès		5/30
		Total	**5/30** (17%)
Pisum sativum	Meknès		19/81
	Fès		3/33
		Total	**22/114** (19%)

* Number of infected samples over total number of samples tested; Percentage of infected samples given in parentheses.

The field symptoms recorded on these species were difficult to define, most likely because of mixed infections. The symptoms consisted of a necrosis of the lower leaves and a mild mosaic on the tip leaves in the case of pea, and vein clearing and mottling in the case of lentil. Infected samples of chickpea revealed a striking wilting of the lower leaves and a yellow mosaic on the upper foliage, while chlorotic spots were observed on common bean leaves. There is no indication that these symptoms are caused by BBMV infection solely.

Discussion

BBMV was transmitted from infected to healthy faba bean by the leaf weevils *Sitona lineatus*, *Smicronyx cyaneus*, *Hypera variabilis*, *Apion radiolus*, and *Pachytychius strumarius*. Among these, *S. lineatus* appeared to be an efficient vector, since an AAP of 15 minutes or the first bite were sufficient for transmission, the rate of transmission was relatively high (c. 41%), and the virus was retained by the insect for at least 7 days. Bawden et al., (1951) already suspected *S. lineatus* to be a vector of BBMV in England, but their transmission tests failed. Much later, Borges and Louro (1974) reported transmission of BBMV by *S. lineatus* var. *viridifrons* Motsch in Portugal at a rate of 5 to 6%. The transmission rate obtained by us appears high, but this discrepancy in data may be due to a difference in weevil biotypes, virus strains, number of plants tested, number of insects per assay plant, and the experimental conditions.

S. lineatus is an important insect pest of faba bean in Morocco, and the adults are encountered there during the whole life cycle of the crop (Chairi, 1989). Although the characteristic feeding damage they cause is not economically important, it now appears that the species is potentially important in the spread of BBMV in nature. Moreover, our results show that this insect is able to transmit the virus from faba bean to the other important food legumes lentil and pea. We could not achieve vector transmission to three breeding lines of chickpea, possibly because the insect does not like feeding on this legume species, but we could detect BBMV in naturally infected chickpea plants.

The transmission efficiency of the other weevils was not evaluated because of the limited numbers of individuals available. Nevertheless, *A. radiolus* seems of potential importance in the transmission of BBMV. A short IAP is enough for the species to transmit the virus. Some *Apion* species have been reported as vectors of BBMV. *A. vorax* has been reported from Britain (Cockbain, 1983) with a transmission rate of 20%, and *A. arrogans* from Syria (Makkouk and Koumari, 1989). Virus transmission by *Apion* spp. has been shown earlier by Cockbain (1971), who found that the weevils of the genera *Apion* and *Sitona* are vectors of broad bean stain and broad bean true mosaic comoviruses. Later, Gerhardson and Pettersson (1974) reported that *A. apricans* Hbst. and *A. varipes* Germ. transmit red clover mottle virus.

This is the first report on *A. radiolus, H. variabilis, S. cyaneus*, and *P. strumarius* as vectors of BBMV, and the first record of the curculionid genera *Hypera*, *Smicronyx*, and *Pachytychius* as virus vectors. This gives further evidence that the members of the Curculionidae are important as virus vectors. Compared to *S. lineatus*, other curculionids are not abundant on faba bean in Morocco, are of no economic importance, but appear now potentially important as vectors of BBMV. Moreover, species of the genera *Apion* and *Sitona* might also be vectors of the comoviruses occurring on faba bean in Morocco, i.e. broad bean stain and broad bean true mosaic viruses, as reported in Britain (Cockbain, 1971). Since BBMV is seed transmitted in faba bean (Fortass and Bos, 1992), the vectors reported here may well play a determinant role in spreading the virus from crop plants infected from seed and from other infected legumes.

Among the 102 samples of the legume weeds *T. alexandrinum, M. scutellata, M. hispida*, and *V. sativa* collected in faba-bean fields, none was found harbouring the virus. Although a large number of legume weeds were found susceptible to mechanical inoculation and were thus reported as potential hosts of BBMV (Makkouk et al., 1988), none of the ones tested in the present study were found to host the virus. More sampling and testing is needed, however. In contrast, common bean and the food legumes pea, chickpea, and lentil were found naturally infected by BBMV. The virus was suspected to occur naturally in food legumes other than faba bean since it has already been found pathogenic to these food legume species and several other fodder and weed legumes upon mechanical inoculation (Makkouk et al., 1988; Fortass and Bos, 1992). Moreover, some isolates of BBMV were found to be more pathogenic on chickpea, lentil, or pea, than on faba bean (Fortass and Bos, 1992), implying therefore that BBMV should be regarded as a food-legume virus rather than a mere faba-bean virus.

This is the first report of the natural occurrence of BBMV in crops of chickpea, lentil, pea, and common bean. Further surveying of food legumes other than faba bean for this virus would undoubtedly reveal its natural occurrence in these crops in other food legume producing regions in the world. Earlier research (Makkouk et al., 1988; Fortass and Bos, 1992) and the present investigations on incidence, natural occurrence in a number of legume crops, and seed and weevil transmission of BBMV in Morocco and other countries in West Asia and North Africa, have clearly shown the virus to be of long-neglected agricultural importance in the region. Moreover, the seed transmission of BBMV in chickpea and pea (Fortass and Bos, 1992) and probably in other legumes, and its transmissibility from faba bean to other food legumes by *S. lineatus*, make this virus a threat to food legume improvement programmes.

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