Relationships Between *Aulacorthum solani* and Soybean Dwarf Virus: Effect of Temperature on Transmission

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ABSTRACT

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Transmission characteristics of two strains of soybean dwarf virus (SDV-D and SDV-Y) were studied using Japanese and California populations of the aphid vector *Aulacorthum* (*Acyrthosiphon*) *solani*. Latent periods of the virus strains were 12 and 15 hr in aphids from the Japanese colony and 15 and 21 hr in aphids from the California colony for SDV-D and SDV-Y, respectively. Both strains persisted longer in the Japanese than in the California aphids and SDV-D persisted longer than SDV-Y in both aphid populations. Both strains had minimal acquisition access (AAP) and inoculation access periods (IAP) of 30 min; optimal AAP and IAP were 48 hr. Transmission and acquisition efficiency of both strains by either vector was greater at 20–22 C than at 29 C, 10–11 C, or 5 C.

Additional key words: luteovirus, subterranean clover red leaf virus.

Soybean dwarf virus (SDV) is the causal agent of a severe disease in soybeans (*Glycine max* (L.) Merr.) in Japan (15). SDV is a luteovirus and like other group members is circulative in its vector and persistently transmitted. Two important strain groups have been described (14) that differ in symptoms, host range, vector relationships, and physiochemical characteristics (7,8,16). The isolates of the dwarfing strain apparently cross protect (13–15).

SDV is of particular interest to our laboratory because of its potential threat to the U.S. soybean industry, a key component of U.S. agricultural production and balance of trade, and because of the similarity of SDV to other important luteoviruses. Subterranean clover red leaf virus (SCRLV), which causes an economically important disease of subterranean clover (*Trifolium subterraneum* L.) in New Zealand and Australia (11,18), is related serologically to the yellowing strain of SDV. Furthermore, there is now evidence that a virus found in California with many characteristics of SCRLV is serologically and morphologically indistinguishable from an Australian SCRLV strain (9). Also, SDV is related serologically to beet western yellows virus (4,5), which has been demonstrated to cause considerable economic damage in agricultural commodities on a worldwide basis.

The most efficient vector of SDV is the foxglove or dock aphid, *Aulacorthum* (*Acyrthosiphon*) *solani* (Kaltenbach) (13). *A. solani* is a nearly ubiquitous, polyphagus aphid that is found in the temperate to cool areas of both hemispheres (10,17). Recent studies in our laboratory have demonstrated several differences in the transmission characteristics of two virus strains (dwarfing and yellowing) with respect to geographically diverse populations of *A. solani* (2). We report here virus strain differences in latent period, virus persistence in aphid populations, minimal and optimal acquisition and inoculation periods, and the effects of temperature on virus acquisition, and inoculation by two divergent populations of the vector.

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MATERIALS AND METHODS

Aphid populations and virus isolates. One *A. solani* population and the dwarfing (SDV-D) and yellowing (SDV-Y) strains of soybean dwarf virus were collected and isolated in Hokkaido by Dr. T. Tamada and hand-carried from Japan to the containment facility at Frederick (2,12) under permits from the Animal and Plant Health Inspection Service (APHIS) and the Maryland Department of Agriculture. A second population of *A. solani*, collected in California was provided by Dr. James Duffus. Colonies of nonviruliferous *A. solani* were established as needed and maintained on *Rumex crispus* L. (curly dock), *Lactuca sativa* L. 'Black-seeded Simpson', or Wayne soybean in acrylic plastic cages in isolation cubicles. Routine maintenance of the aphid colonies and the virus isolates was described earlier (2,7).

Each transmission experiment was done with both the Japanese and California aphids and with SDV-D and SDV-Y. Unless otherwise noted, the following procedure was used for transmission studies. Several Wayne soybean trifoliolates exhibiting symptoms of either SDV-D or SDV-Y were separated into individual leaflets. The leaflets were placed in petri dishes in such manner that each dish was a comparable virus source. Nonviruliferous, late instar, or adult *A. solani* were allowed a 48-hr acquisition access period (AAP) on the leaflets before transfer to 1-wk-old unifoliolate Wayne soybean seedlings (VC stage [6]). After a 48-hr inoculation access period (IAP), the aphids were killed with malathion spray (0.02% active ingredient per liter). Thirty minutes later the plants were sprayed with distilled water to reduce phytotoxicity and moved to greenhouse benches under natural light (14 hr average) at 23–27 C. Plants were observed daily and disease symptoms recorded after 20 days. Control inoculations were made with aphids, which were allowed a 48-hr AAP on uninfected tissue, then transferred to soybean seedlings. All experiments were repeated at least twice and all treatments were

replicated at least five times.

Latent period. The latent period, defined here as time elapsed from the start of the AAP until the end of the IAP when aphids transmitted the virus, was determined. In the first series of experiments, late instars or young apterous adults were allowed a 3-hr AAP, then starved on moist filter paper in petri dishes. At 3-hr intervals, for 21 hr, five randomly selected aphids were placed onto each of 10 test plants for a 3-hr IAP. In a parallel group of experiments, the aphids were allowed continuous access to the same virus source but were not starved. At 3-hr intervals for 21 hr, groups of five aphids were removed from the virus source and placed onto test plants for a 3-hr IAP. The aphids from both experimental series were then killed with malathion spray and placed on greenhouse benches for observation. All test plants were observed for symptoms and latent periods calculated. To ensure that the environmental conditions, the virus sources, and the aphid populations were in satisfactory condition, control transmission tests were done. Additional aphids were allowed a 48-hr AAP on each virus source, after which groups of five were transferred to each of 10 test seedlings for a 48-hr IAP. At the end of each control experiment, transmission efficiency was determined for each aphid population with each virus source.

Virus persistence. Persistence of SDV-D and SDV-Y in individual apterae of Japanese and California aphids was determined through sequential 24- or 48-hr transfers of single aphids to individual test plants following a 72-hr AAP. All combinations of aphid populations and virus strains were tested with 40 aphids per combination. Transfers continued until the death of all test aphids. Transmission data were recorded daily for each aphid. Test plants were held for observation for 21 days.

Minimal and optimal acquisition and inoculation periods. Nonviruliferous aphids were allowed access to a virus source for 1/2, 1, 6, 12, 24, and 48 hr. At the end of each time period the aphids were transferred in groups of five, to 12 test plants, for IAPs of 1/2, 1, 6, 12, 24, and 48 hr. All combinations of AAP and IAP were tested with the two aphid population with the dwarfing strain.

To maximize transmission efficiency, all combinations of 24, 48, 72, or 96 hr AAP and IAPs were tested with both virus strains to determine the optimal AAP and IAP. The experiment was replicated 24 times and repeated three times.

Temperature. The effects of high (20–22 C), moderate (10–11 C), and low (5 C) temperatures on virus acquisition were tested. After a 24- or 48-hr acquisition access in environmentally controlled growth chambers at the above temperatures, groups of five aphids were transferred to uninfected soybean seedlings for a 48-hr IAP at 22 C.

Temperature effects on inoculation were measured in the growth chambers. Nonviruliferous *A. solani* were allowed a 48-hr AAP on infected leaves at 20 C and transferred in groups of five to soybean seedlings. After a 48-hr IAP at 10–11 C, 20–22 C, or 29 C, the aphids were killed and the test plants moved to the greenhouse for observation.

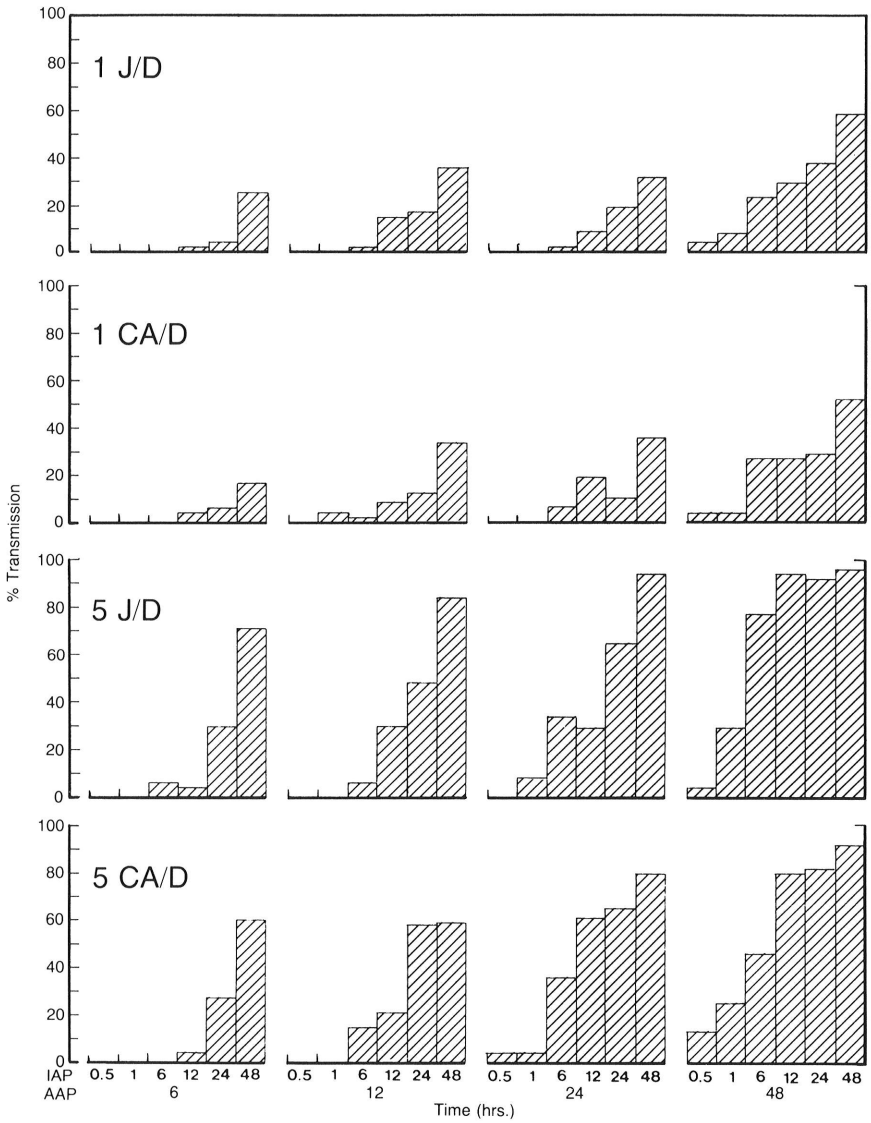

Fig. 1. Effect of 1/2- to 48-hr acquisition access and inoculation access periods on transmission and time to symptom development of the dwarfing strain of SDV by one and by five Japanese or California apterae of *Aulacorthum solani*.

RESULTS

Latent period. Regardless of the method used, the latent period in the Japanese *A. solani* was shorter than in the California *A. solani*. When the aphids were allowed to feed on the virus source for only 3 hr and then starved, the latent period for both SDV-D and SDV-Y was 12 hr in the Japanese population. With the California population, however, the latent periods for SDV-D and SDV-Y were 24 and 30 hr, respectively (data not shown). If the AAP was continuous until immediately before a 3-hr IAP, the latent periods of SDV-D and SDV-Y were 12 and 15 hr, respectively, in the Japanese population and 15 and 21 hr in the California population (Table 1). When control aphids that had been allowed 48-hr AAPs were transferred to test seedlings for 48-hr IAPs, the Japanese apterae transmitted SDV-D and SDV-Y to 95 and 88% of the seedlings, respectively, whereas the California apterae transmitted SDV-D and SDV-Y to 97 and 80% of the test seedlings.

Virus persistence. After a 72-hr AAP, both virus strains were transmitted by both aphids within 24 hr of transfer to test seedlings. However, transmission efficiency increased daily for the first 3–5 days following the AAP (6–8 days after AAP initiation), then decreased thereafter. With any combination of aphid and virus, the transmission pattern became erratic as the time after acquisition increased. The Japanese *A. solani* transmitted SDV more efficiently than the California aphids and SDV-D was transmitted with greater frequency than SDV-Y by both vectors (Table 2). Infestation with SDV-D or SDV-Y had no effect on the mean survival of either vector but the Japanese aphids survived for an average of 4 more days on the host plants than the California aphids.

Minimal and optimal acquisition and inoculation access periods. Transmission of SDV was affected by the length of both the AAP and IAP (Fig. 1). No transmission of SDV-D was observed with single California or Japanese aphids with AAPs of less than 6 hr; however, acquisition occurred within 30 min with sets of five Japanese aphids and within 1 hr with sets of five California aphids. Within each AAP, transmission percentages increased (Fig. 1) as IAPs increased from 1/2 to 48 hr. Increasing the AAP also increased the percent transmission. Similarly, in experiments where the AAP or IAP increased from 24 to 96 hr, the number of plants infected by either virus strain also increased. No measurable increase in transmission was observed if the AAP and IAP were both greater than 48 hr. Regardless of the number of aphids used, symptoms of SDV-D and SDV-Y were observed 5–8 days and 11–14 days after inoculation, respectively.

Temperature effects. After a 48-hr AAP at all temperatures tested, SDV-D and SDV-Y were transmitted by both the Japanese

TABLE 1. Latent period[a] of the dwarfing (D) and yellowing (Y) strains of soybean dwarf virus (SDV) in adult apterae of *Aulacorthum solani* from California (CA) and Japan (J)

Aphids (no.)	Vector/ strain	Latent period (hr)						
		6	9	12	15	18	21	24
5	J/D	0/8	0/8	*1/18*[b]	2/18	1/28	4/14	7/18
5	CA/D	0/8	0/8	0/38	*1/38*	1/48	7/48	5/48
5	J/Y	0/8	0/8	0/18	*1/18*	2/27	5/18	5/18
5	CA/Y	0/8	0/8	0/48	0/38	0/38	*1/48*	2/48

[a] Latent period is defined as elapsed time from the start of the acquisition access period until the end of the inoculation access period when aphids transmitted the virus. Acquisition access period was 3–21 hr followed immediately by an inoculation access period of 3 hr.

[b] Italicized data indicate earliest transmission for the virus/vector combination.

TABLE 2. Persistence of soybean dwarf virus (SDV)-dwarfing (D) and SDV-yellowing (Y) in late-instar nymphs or adults after a 72-hr acquisition access period on infected Wayne soybean

Variables	*A. solani* (Japan)		*A. solani* (California)	
	SDV-D	SDV-Y	SDV-D	SDV-Y
Total seedlings infected/total seedlings tested	168/381 (44.1%)	113/294 (38.4%)	92/311 (29.6%)	30/258 (11.6%)
Number of aphids that transmitted virus/total number of aphids	35/40	29/40	34/40	14/40
Average number of transmissions/aphid	4.8	3.9	2.7	2.1
Most transmissions by a single aphid	10	10	6	6
Longest retention by a single aphid (days)	23	23	10	9
Longest consecutive transmission by a single aphid (days)	9	7	6	6
Mean survival of viruliferous vectors (days)	12.6	9.6	7.5	6.7
Mean survival of nonviruliferous vectors (days)	11.1		7.1	

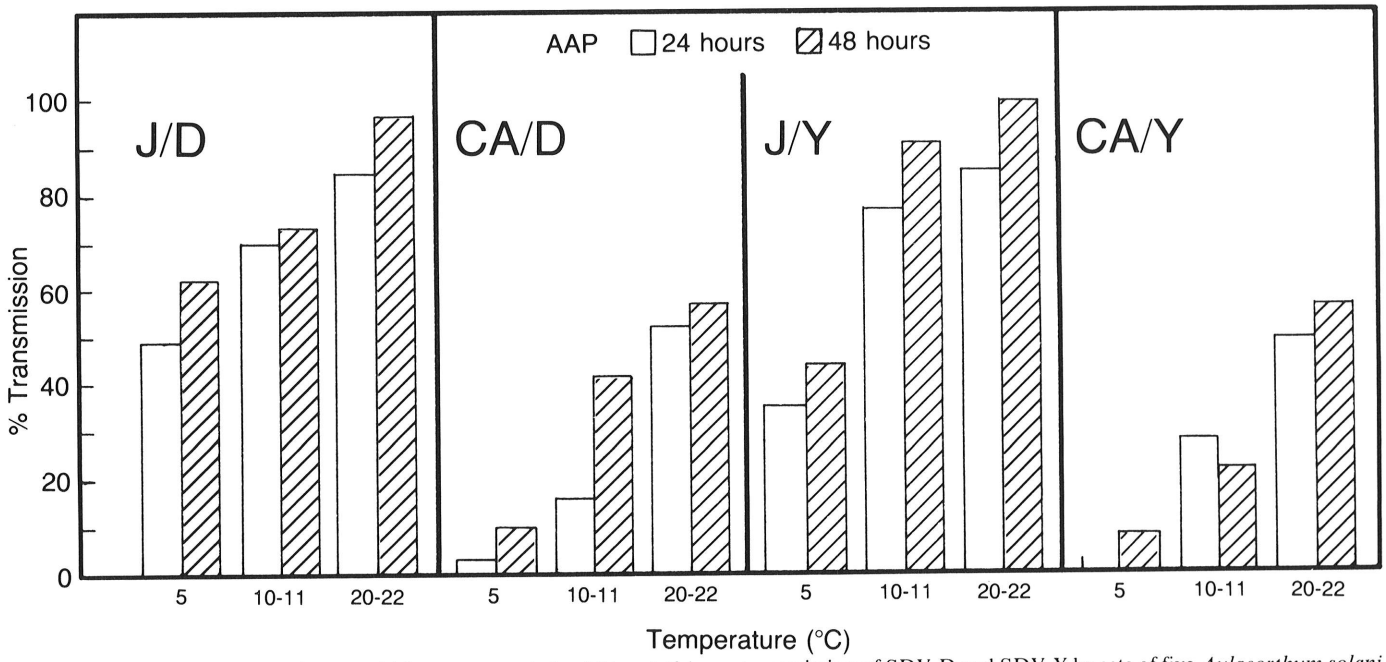

Fig. 2. Effect of temperature during acquisition access periods of 24 and 48 hr on transmission of SDV-D and SDV-Y by sets of five *Aulacorthum solani* apterae. Temperature during inoculation access was 20–24 C.

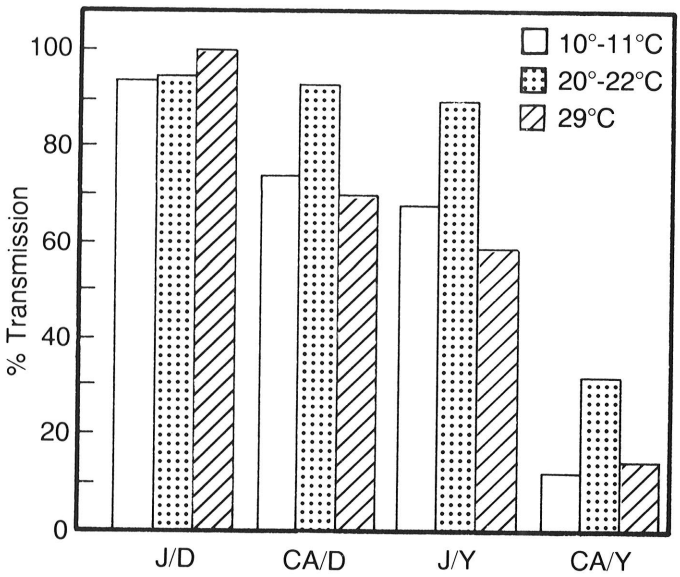

Fig. 3. Effect of temperature during 48-hr inoculation access periods on transmission of SDV-D and SDV-Y by sets of five *Aulacorthum solani* apterae. Temperature during acquisition was 20 C.

and California aphids (Fig. 2). As the AAP temperature increased, both populations acquired and subsequently transmitted both strains more efficiently. At each temperature tested, the California population transmitted both SDV-D and SDV-Y less efficiently after a 48-hr AAP than the Japanese population transmitted the viruses after a 24-hr AAP (Fig. 2). Transmission at all temperatures was more efficient after a 48-hr than after a 24-hr AAP.

Temperature had a greater effect on transmission of SDV-Y than on SDV-D (Fig. 3), and more effect on the California aphids than on the Japanese aphids. Transmission of both strains was most efficient at 20–22 C.

DISCUSSION

Differences exist in morphology and feeding preferences of populations of *A. solani* from different areas of the world (2,3). The aphid has several primary hosts and peak populations of the major migratory generation usually occur between early June and late July in the Northern Hemisphere (10,17). Wave et al, in a study of the life cycle and distribution of *A. solani* in the northeastern United States, found that the viviparae reach their greatest density in mid-August (17). Populations of the aphid exist in nature (1,2,17) that differ in primary and secondary host preference, anatomical characteristics, and transmission efficiency. Geographically separated *A. solani* populations also transmit SDV with differential efficiency.

Latent period, persistence, and transmission efficiency are characteristics that are influenced by the vector population, the host species and cultivar, and environmental conditions. Persistently transmitted viruses typically have a latent period in the vector of at least 12 hr, can be retained by the vector through molts, and a lengthy AAP and IAP is required for efficient transmission. SDV is no exception. Tamada (13) found that SDV had a latent period in the vector of 15–27 hr which is typical of a luteovirus and consistent with the 12–15 hr latent period for SDV-D and 15–21 hr for SDV-Y reported here. Tamada also found that SDV persisted in the Japanese vector up to 21 days with a gradual loss of infectivity in later transfers (13). We found that SDV-D persisted in the Japanese vector for up to 23 days but only for 10 days in the California vector. In both populations an AAP and IAP of at least 48 hr was required for optimum transmission efficiency.

Several differences were observed in the transmission characteristics of the two virus strains and the two aphid vectors. Regardless of the parameter, SDV-D was transmitted more efficiently than SDV-Y by both *A. solani* populations; the virus persisted in the vectors for a longer period and the percent transmission was greater. Also, the Japanese *A. solani* were more efficient vectors of both strains. This is to be expected because the Japanese population readily colonizes soybean in the field and the California population does not. However, the mean survival of the California *A. solani* on soybean was 7.1 days, suggesting that in the absence of more suitable hosts, the vector might infest soybean for limited periods.

Temperature affected the acquisition and transmission of both strains by both aphid vectors. *A. solani* can be reared successfully between 5 and 25 C (*unpublished data*) and optimally at 10–15 C. Within the temperature ranges tested for acquisition (5–22 C) and transmission (10–29 C) the effects seen in Figures 2 and 3 were probably due to increased feeding activity of the vector.

When many aphids (20 or more) were allowed to feed on soybean seedlings for longer than 48 hr there were fewer plants infected than when only 5–10 aphids were used for inoculation. Under conditions of heavy aphid pressure, a delay in symptom development was observed. One possible reason for this trend is that *A. solani* has a phytotoxic saliva (2,17) that causes rapid necrosis of host tissue at the feeding site giving the aphid a well-earned reputation as a pest in its own right (17). Feeding by a large number of aphids may damage leaf tissue sufficiently to physically inhibit translocation of the virus resulting in reduced systemic infection.

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