Effect of Temperature on Mottling of Soybean Seed Caused by Soybean Mosaic Virus

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ABSTRACT

Temperatures during flowering and especially early pod development were the most influential in affecting the development of mottling of seed from soybean mosaic virus (SMV)-infected plants of moderately and highly susceptible soybean genotypes. Exposure of infected plants to 20 C during this growth period caused the greatest percentage of seed coat mottling on both genotypes, whereas, exposures to 30 C significantly reduced mottling of seed of the susceptible genotype and virtually eliminated this symptom on the moderately susceptible cultivar. Nonmottled and mottled seed from SMV-infected plants transmitted the virus equally. Phytopathology 60:1798-1800.

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Before the relationship between soybean (*Glycine max* L. Merr.) seed coat mottling and soybean mosaic virus (SMV) infection was demonstrated (2, 3, 5), investigators recognized that seed coat mottling was influenced by hereditary traits and environmental factors (1, 4, 11). Observations of the earlier investigators, however, were probably confounded by differences in SMV distribution and/or transmission among experimental plants. More recently, seed mottling varied with location and season in artificially inoculated plants (7, 9), and with time of planting in naturally infected plants (2).

The development of pigments in soybean seed coats is caused by the accumulation of flavoid compounds such as anthocyanins (3, 4). Since the formation of these compounds responds to temp changes (10), experiments were conducted with SMV-infected soybeans grown to maturity at various temp to determine the response of pigment formation in the seed coats.

MATERIALS AND METHODS.—Carborundum-dusted primary leaves of soybean seedlings were inoculated by rubbing with cotton swabs saturated with a suspension of isolate SMV-1 (8). Two soybean genotypes were used: D65-5299, an experimental line highly susceptible to seed mottling (supplied by E. E. Hartwig); and cultivar Lee, moderately susceptible to seed mottling.

Plants were grown either in temp-controlled greenhouse chambers or in growth chambers in the Southeastern Plant Environmental Laboratories at N.C. State University. For the growth chamber experiment, Lee and D65-5299 seedlings were grown in a gravel-peat moss medium in 900-ml plastic containers under 15-hr photoperiods in the greenhouse (28 C) until primary leaves were expanded. Inoculations were made with a purified SMV-1 preparation (6). Plants were then placed in growth chambers, illuminated daily for 9 hr, 4,000 ft-c, and irrigated periodically with a modified Hoagland's solution. Plants were grown at 20, 25, and 30 C during four growth periods: (i) from inoculation to flowering; (ii) from initiation to cessation of blooming; (iii) early pod set (EPS), from cessation of flowering until pods were approx one-half filled; (iv) late pod set (LPS), from one-half pod filling until plant maturity. At the end of the growth periods, plants were moved from one temp (chamber) to another. There were 45 treatments, each containing two plants of each genotype.

Data on seed mottling were obtained by placing individual seeds into categories on the basis of the percentage of seed coat mottled. The mottling categories for Lee were 0%, 0-5%, 5-20%, 20-40%, and 40% and over; and for D65-5299 were 0%, 0-25%, 25-50%, 50-75%, and 75-100%. An average percentage mottling for each plant was obtained by summing the products obtained by multiplying the number of seed in each category by the median category value (37.5 for 25-50% category), then dividing this sum by the total number of seed from the plant.

The percentage SMV transmission was obtained by planting the seed after they were graded for mottling, and was based on the number of infected seedlings of those that emerged. The number of seed planted from each treatment of the growth chamber experiment varied from 26-103 (average 61) for Lee to 4-69 (average 39) for D65-5299.

RESULTS.—In a preliminary experiment conducted in a temp-controlled greenhouse during the summer, two groups of 12 SMV-infected plants of D65-5299 were grown to maturity, one at approx 21 C, the other at 32-45 C. Seed from plants grown at 21 C were heavily mottled, whereas those from the higher temp were only slightly mottled. SMV transmission by seed produced at the low and high temp was 62 and 47%, respectively.

Data from the growth chamber experiment showed that temp during EPS had the greatest influence on seed coat mottling; the cooler the temp, the greater was the percentage of seed-coat mottled (Table 1). The effect was most pronounced in seed of Lee, which

TABLE 1. Effect of temp during early pod set (EPS) on the percentage mottling of seed from cultivar Lee and D65-5299 soybean infected with soybean mosaic virus

Temp during EPS	% Seed coat mottled[a]	
	Lee	D65-5299
C		
20	13.0 b	70.8 e
25	1.5 c	51.0 f
30	0.2 d	36.5 g

[a] Values are averages derived from seed from 8 to 18 plants. Within each genotype, values with different letters are statistically different at the 5% level.

had virtually no mottling when plants were grown at 30 C during EPS, and 13.0% when grown at 20 C during this period. One anomalous result was that only 5% mottling occurred on seed from Lee plants grown at a constant 20 C; this was less than half that on seed from any other 20-C EPS treatments. There was also a trend for a greater percentage of seed mottling to be associated with exposure to 20 C during blooming than was associated with exposures to higher temp (Table 2).

Temperature during the LPS influenced the color of the mottled areas of the seed coat. Greyish-black colors developed on seed exposed to 20 C, whereas, brownish pigments developed on seed exposed to 30 C during this period.

Temperature did not alter the percentage of seed transmission of SMV. Transmission in seed from replicate plants in certain treatments varied widely. The highest percentage transmission was 76.9 and 39.8% in seed of D65-5299 and Lee, respectively.

To determine whether seed transmission of SMV is associated with seed mottling, seed from nine Lee plants having large amounts of mottling were grouped into classes having the same percentage of their seed coat mottled. The seed were planted, and the percentage of virus transmission was based on infected seedlings among emerged plants. Virus transmission was 27, 13, 17, 25, and 35% for groups of seed with 0, 1-5, 5-20, 20-40, and 40% + of their seed coat mottled, respectively.

To verify the above effects of temp on seed mottling, SMV-infected plants of D65-5299 and Lee were grown outside the greenhouse during the summer in 51-cm pots. Two weeks after flower initiation, plants were moved nightly into 15- or 27-C chambers and were placed outside during the days. These exposures lasted 5 weeks, and each treatment contained two plants of each genotype. Control plants remained outside continuously at an average min night temp of 22 C (17-24 C) during the exposure period. For D65-5299 maintained at night temp of 15 and 27 C, 32.5 and 11.7% of the seed coats, respectively, were mottled; seed from control plants had 23.9% mottling. With Lee, 6.6, 2.0, and 1.3% of the seed coats were mottled from the 15- and 27-C exposures and the controls, respectively. Differences between each treatment and the control were statistically significant for D65-5299, but not for Lee.

TABLE 2. Effect of temp during blooming on the percentage mottling on seed from cultivar Lee and D65-5299 soybean infected with soybean mosaic virus

Genotype	Temp during early pod set	% Seed coat mottled[a]		
		Temp during blooming		
		20	25	30
	C			
Lee	20	19.0	16.0	12.8
D65-5299	20	80.9	66.9	76.4
Lee	25	3.2	0.5	0.7
D65-5299	25	55.9	47.7	34.9
Lee	30	0.2	0.3	0.1
D65-5299	30		45.1	36.6

[a] Values are based on seed from 6-12 plants.

DISCUSSION.—These experimental results indicate that development of anthocyanins in seed coats of SMV-infected soybeans can be greatly affected by temp. Results from the growth chamber experiment verified those from the greenhouse experiment, and indicated that the most formative period for the temp effect on seed mottling is during the early development of the fruit. Since soybean flowers open sequentially over a period of weeks, there is considerable difference in pod development on each plant during this time. Hence, when plants were moved to or from the EPS temp, pods were in various stages of development. This may explain some of the variation in mottling of seed from individual plants.

When soybeans are grown under artificial environments, such as in greenhouses or growth chambers, flowers may undergo fertilization and pods initiate before blooms appear (petal exposure). Such a delay in petal exposure on plants at 20 C would have delayed the transfer of the plants to a higher temp. This additional exposure to 20 C during the early development of some pods could have caused the slight increase in mottling attributed to 20-C exposures during blooming (Table 2).

The relatively low amount of mottling developed on seed from Lee plants maintained at 20 C throughout, compared with that developed on seed from plants placed at 25 or 30 C either before or after an EPS of 20 C, indicates that with certain genotypes, temp higher than 20 C at some time during the life of the plants may be necessary for full pigment development.

Formation of anthocyanins has been associated with temp between 15 and 20 C, and results of the present experiments agree in general with data on isoflavone formation in subterranean clover (*Trifolium subterraneum* L.) (10).

Results of the seed transmission tests indicated that the temp used had little or no effect on virus transmission, and that the percentage of virus transmission in seed cannot be predicted from the amount of seed coat mottling. The latter verifies conclusions from Japan (3), but disagrees with results of previous field work showing greater SMV transmission from mottled seed than from nonmottled seed (7, 9). The discrepancy may have occurred either because occasional field plants escaped

infection and produced nonmottled seed, or because of environmental differences during seed drying. Since SMV can be recovered from almost all immature seed, but is recovered with increasing difficulty as seed mature, the Japanese have postulated that this and the usual low percentage of seed transmission are due to virus inactivation during maturity and seed drying (3). In commercial seed lots from fields with less than 100% infection, removal of mottled seed would reduce SMV seed transmission, since mottled seed occurs only on infected plants.

The alteration in percentage mottling of seed from D65-5299 grown outside the greenhouse and exposed to cool or warm night temp shows that the thermal response occurs under conditions more natural than those of the greenhouse and growth chamber. With plants grown outside, mottling of Lee seed was not as responsive as that of D65-5299 to the night temp.

The influence of temp on soybean seed mottling from SMV-infected plants may be responsible for the attention given the problem in the North (1, 2, 4, 11). In Minnesota, more mottling occurred on seed from plants sown on 1 July than on those sown earlier (2). Whereas, these results could have been caused by greater viruliferous vector populations later in the season, so that plants sown on 1 July became infected while younger than those sown earlier (as mentioned by the authors), they also could have resulted from later flowering and cooler temp during EPS than prevailed for plants sown earlier.

The responsiveness of the young fruit to environmental conditions and its effect on mottling of the mature seed appears related to previous information, showing that immature seed from SMV-infected plants contain compounds that become red when the seed are boiled in methyl alcohol (3). These pigment precursor compounds, and their transformations are apparently quite susceptible to prevailing environmental conditions and appear to govern the degree of mottling of the mature seed.

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