Barley Stripe Mosaic Virus Infection of Corn and the "Aberrant Ratio" Genetic Effect

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ABSTRACT

Barley stripe mosaic virus (BSMV) infection and systemic invasion were studied in a corn line previously found to exhibit altered progeny ratios if infected with BSMV and used as a male parent in crosses. This phenomena had been designated "Aberrant Ratio" (AR). Substantial quantities of single-stranded viral RNA (21 S) were detected in photometric scanning patterns from density-gradient centrifugation of cellular nucleic acids extracted from infected corn leaves 3-7 days after inoculation, but intact viral nucleoprotein production during this period was very low. In contrast, systemically invaded tissue of leaf two of the BSMV-resistant 'Moreval' barley produced a lower viral RNA yield and a higher nucleoprotein yield than did the corn variety.

Single-stranded and replicative-form RNA were detected in corn tassel meristems of infected plants by in vivo ^{32}P-labeling. Both viral RNA species were apparent from 13 days after inoculation until tassel emergence. No evidence of pollen or seed transmission of BSMV was detected in progeny of selfed, infected plants. Attempts to detect viral RNA in plants with the AR genetic effect were negative. The ND 18 strain of BSMV induced the AR effect, but no evidence of intact viral RNA species was found in the progeny.

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Additional key word: mutation.

A fundamental question concerning the effect of plant virus infection on the genetic system of its host has been posed by the results of Sprague et al. (17) and Sprague and McKinney (15, 16), who described a genetic abnor-

mality of corn induced by barley stripe mosaic virus (BSMV) infection of a pollen parent line. If the infected pollen parent, A_1A_1, PrPr, SuSu (A_1-a_1, presence or absence of aleurone color, Pr-pr, purple or red aleurone color; Su-su, starchy or sugary seed, hereafter designated male line) was crossed with a homozygous recessive line (a_1a_1, prpr, susu, resistant to BSMV), a low frequency of the progeny lines produced significant distortion from expected ratios for one or more of the genetic markers. This effect has been designated "Aberrant Ratio" (AR), and was apparent only if the original pollen parent was virus-infected and exhibited systemic symptoms of infection in upper leaves.

A number of explanations, including an influence of gametophyte factors and chromosomal aberrations have been excluded (15), in that AR is characterized by essentially equivalent distortion of progeny ratios through both female and male transmission. Unusual genetic behavior of AR suggests that the AR-related agent is regulatory; the agent is nuclear, but does not show characteristics of normal chromosomal behavior (15), and is capable of transposition from one locus to another (16). The effect can be transferred from a virus-exposed gene to its nonvirus-exposed allele, presumably by crossing over (15). The AR effect is inherited in a stable manner in the affected lines, although the distorted ratios can, at low frequency, revert to normal segregation patterns (15). Even though there is no evidence of the presence of an infectious agent in the progeny (15, 17), it may be possible that a portion of the viral genome is present in the affected plants. Wheat streak mosaic virus and lily fleck corn virus also elicit the AR effect (16). Wheat streak mosaic virus and BSMV contain RNA as their nucleic acid (2, 9).

The objectives of this study were to characterize the early events of infection of the male corn line by the use of in vivo ^{32}P-labeling, to establish the extent of systemic invasion of the male line by BSMV or BSMV-RNA, and to search for alien nucleic acid species in the AR progeny. For comparative purposes, a BSMV-resistant barley cultivar ('Moreval', C.I. 5724) was included in experiments concerned with early events of infection. Procedures designed to detect the synthesis of both single- and double-stranded viral RNA (9, 10) were employed. In addition, experiments were designed to determine if other strains of BSMV would also produce the AR effect.

MATERIALS AND METHODS.—*Culture of plants.* — Seed of the corn (*Zea mays* L.) lines was planted in 10.2-, 15.2-, or 30.5-cm diam pots containing composted, autoclaved soil. The pots were then placed in growth rooms at 17, 25, or 32 C, with incandescent and/or fluorescent (cool-white) light producing about 12,912 lx (1,200 ft-c) light intensity at plant height for 12 or 16 hr/day. Greenhouse-grown plants were maintained at 22-27 C under natural day-length periods.

Culture of virus.— The Argentina mild isolate of BSMV (received from H. H. McKinney, Beltsville, Md.) and the ND18 culture of BSMV were maintained by weekly transfer in 'Black Hulless' (C.I. 666) barley (*Hordeum vulgare* L.) at 25 C. Two other strains of BSMV (ATCC 69 and ND110) were used in preliminary experiments. Plants were inoculated by manual leaf rubbing using Celite (Johns-Mansville Co., diatomaceous silica).

Purification of BSMV.— Freshly harvested corn or barley tissue was ground with a mortar and pestle or a Waring Blendor with about five times its weight of 0.5 M sodium orthoborate buffer (pH 9.0). The extract was expressed through two layers of cheesecloth and centrifuged at 7,700 *g* for 20 min. The supernatant was then centrifuged at 92,000 *g* (28,000 rpm) for two hr in a No. 30 rotor (Beckman Instruments, Inc., Palo Alto, Calif.). The pellets were resuspended in 0.1 M potassium phosphate buffer, pH 6.0, containing 0.1% Igepon T-73 (sodium *N*-methyl *N*-oleoyl taurate, General Aniline and Film Corp., New York) and centrifuged at 7,700 *g* for 20 min. The supernatant was layered over 15 ml of 200 mg sucrose/ml containing 0.1% Igepon T-73 and centrifuged for three hr at 92,000 *g* (28,000 rpm) in a Beckman No. 30 rotor. The resultant pellets were resuspended in 0.1 M potassium phosphate buffer (pH 6.0). Density-gradient centrifugation of the preparation was the same as previously described (9).

Preparation of nucleic acids.— Total cellular nucleic acids were extracted as previously described (9). In vivo ^{32}P-labeled nucleic acids were prepared by 6-18 hr uptake of 0.5 mCi $H_3\,^{32}PO_4$ (New England Nuclear, carrier-free) before extraction. The replicative form (RF) of BSMV-RNA was prepared by ethanol precipitation of the 2.0 M LiCl-soluble nucleic acids, followed by digestion of DNA and single-stranded RNA with deoxyribonuclease and ribonuclease (10).

Nucleic-acid preparations were analyzed in linear-log sucrose-density gradients designed for the Beckman SW41 rotor (1). Gradients were made in 0.15 M sodium chloride, 0.015 M sodium citrate (SSC), pH 7.0, and were allowed to diffuse 12-15 hr at 4 C before use. About 40 μg of total nucleic acids were layered on each gradient, and the tubes were centrifuged at 259,000 *g* (39,000 rpm) for 5.0-8.0 hr at 14 C in the Beckman L265B ultracentrifuge. The rotor was allowed to come to rest without braking. Fractionation of gradients and radioactivity determinations were made as previously described (9).

RESULTS.—*BSMV infection development in corn.* — Several corn varieties ('Golden Giant', 'Golden Cross Bantam', 'Ohio 28', 'Nebraska 501D', and the male line) were inoculated with four strains [type (ATCC 69), ND110, ND18, and Argentina mild] of BSMV under three temperature regimes (17, 25, and 32 C). In general, a higher percentage of inoculated plants became infected at 32 C than at 17 or 25 C. Symptom severity was greater at lower temperatures. The Argentina mild and ND18 isolates were more virulent in the male line than the other isolates; high percentages of infection developed at 25 or 32 C. Systemic invasion of the male line was better with the ND18 strain than with Argentina mild. The ND18 strain was selected for experiments designed to induce persistent systemic symptoms.

Symptoms of ND18 infection of the male line first appeared in leaves that emerged 4-5 days postinoculation (DPI). A severe mosaic was apparent in the two or three newly emerging leaves (Fig. 1-A). As new leaves continued to emerge, a coarse mottle often became the predominant symptom. Long stripes, 10- to 25-mm wide, or spindles developed in the upper leaves if plants were grown at 25 C under 12-16 hr daylength. The nature of the striping pattern indicated that the progeny of a few meristematic blade cells became infected early in their development, and very little lateral spread of the virus occurred in mature leaves. Infectivity assays were conducted with excised spin-

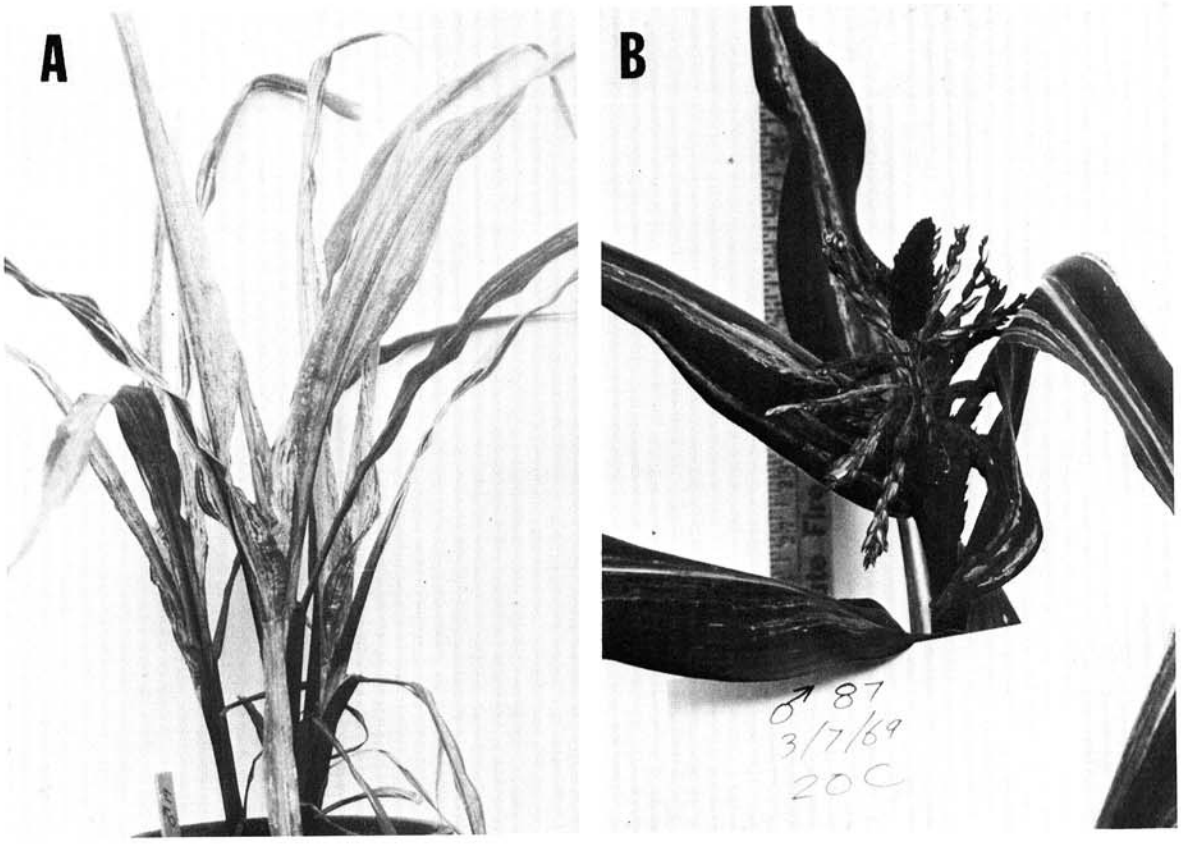

Fig. 1. Symptoms of barley stripe mosaic virus (BSMV) infection of the male-line corn plants grown at 20 C under fluorescent light. **A)** Severe mottle-mosaic of systemically invaded leaves 32 days postinoculation (DPI). **B)** Tassel and symptoms on upper leaves and flag leaf of BSMV-infected corn.

dles or streaks from full-length leaves; it was difficult to demonstrate infectivity from these tissues. Purification experiments revealed a very low (ca. 10 μg/g) concentration of BSMV in these areas. Few plants had systemic symptoms extending to the terminal leaves if grown in the greenhouse, or at 25 C under incandescent and fluorescent light. The difficulty in producing systemic invasion of BSMV-infected corn has been previously described (8, 17), and the present results confirm those studies. Greenhouse and growth-room observations indicated that the rapidly elongating plants simply outgrew the slowly spreading virus, and upper leaf meristems differentiated without becoming invaded.

An attempt was made to produce systemic symptoms by growing the plants under suboptimal conditions. Plants were grown singly or three per 15.2- or 30.5-cm diam pot at 25 C under fluorescent light. Ten days after inoculation, half of the pots were transferred to a growth room at 20 C under fluorescent light. Each pot was fertilized with commercial 20-20-6 fertilizer once per wk. Plants grown at 25 C under incandescent and fluorescent light elongated rapidly, probably as a result of the increased far-red illumination from incandescent light. Plants were about 2 m in height at maturity, and few showed symptoms in the upper six-to-eight leaves. Greenhouse-grown plants behaved similarly.

Observations made over the entire growth period indicated that plants grown at 20 C under fluorescent light in small pots grew slowly, and many (25-30%) produced excellent systemic symptoms in all leaves, including the flag leaf. The plants were 60- to 90-cm tall at maturity and produced large tassels (Fig. 1-B), which shed copious amounts of viable pollen. Ear shoots were seldom produced, and attempts to self the infected plants were usually futile, in that the plants rapidly became senescent after anther exsertion. Nevertheless, 96 seeds from selfed, infected plants with symptoms on uppermost leaves were collected from 16 plants. None of the plants grown from these seeds displayed symptoms of BSMV infection. Sib matings were also made involving pollen donors with systemic symptoms and emasculated, field-grown uninfected pollen-acceptor plants. About 200 plants grown from seed of this cross did not produce symptoms. Thus pollen or seed transmission of BSMV in corn was negative, as has been reported (15, 17), although the limited number of test plants used in this study does not preclude the possibility of seed or pollen transmission in a very low percentage.

ND18 BSMV as an inducer of the AR effect.— Plants of the pollen-donor line (A_1A_1, PrPr, SuSu) were grown singly or three per 15.2-cm diam pot at 25 C as described above. At 10 days after planting, the plants were inoculated with ND18 BSMV and were transferred to 20 C when

symptoms appeared. The virus culture used in this experiment was selected from upper corn leaves of three successive transfers through the male line in an attempt to select a population of the virus adapted for systemic invasion. Pollen acceptor plants (a_1a_1, prpr, susu) were grown singly in 30.2-cm diam pots in a greenhouse. Each plant was emasculated as the tassel emerged. Pollen was collected from infected plants and used to fertilize the homozygous recessive plants. Seed was collected and sent to G. F. Sprague at Beltsville, Maryland for appropriate testing. Genetic analyses to date indicate at least one verified AR case in the progeny (G. F. Sprague, *personal communication*).

Viral RNA and nucleoprotein production in the male corn line and in BSMV-resistant Moreval (C.I. 5724) barley.—The early events associated with ND18 BSMV infection of the male corn line were monitored as previously described for BSMV infection of Black Hulless barley (9). Freshly harvested tissue was used for purification of intact virus and for extraction of cellular nucleic acids. The BSMV-resistant Moreval (C.I. 5724) barley variety was included for comparison. The second and third leaves of the male corn line were inoculated at 10 days after planting with ND18 BSMV from Black Hulless barley inoculated 10 days previously. Leaf one of Moreval barley plants was inoculated at seven days after planting with inoculum from a 10-day infection of Moreval barley. Beginning at two days after inoculation, leaves were harvested daily until seven days after inoculation. Plants from which leaves had been harvested were saved; symptom development in newly emerging leaves indicated that nearly all inoculated plants became infected.

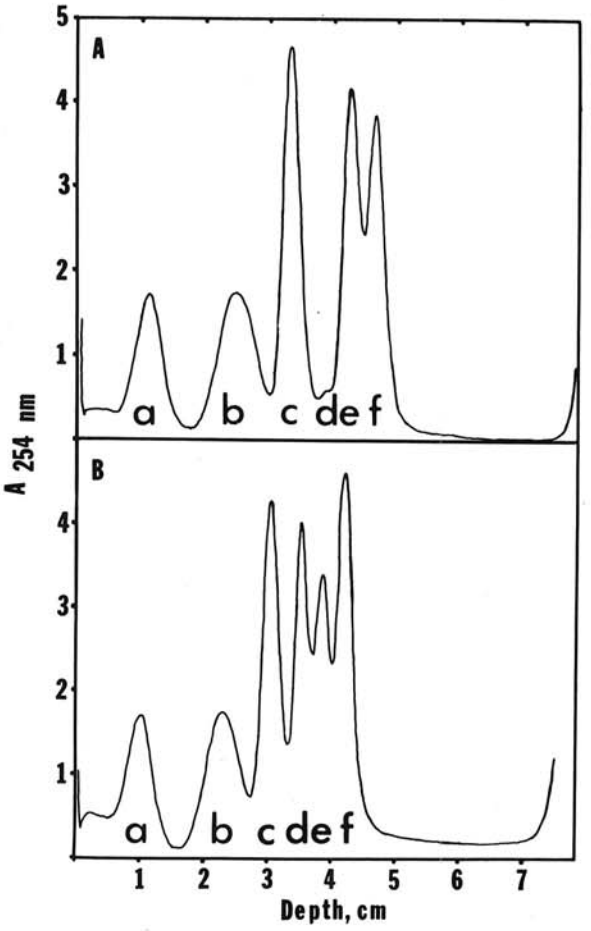

Fig. 2. Photometric scanning patterns of nucleic acids subjected to density-gradient centrifugation on linear-log sucrose gradients in the Beckman SW 41 rotor. **A)** Nucleic acids of healthy male-line corn. **B)** Nucleic acids from barley stripe mosaic virus-infected male-line corn, including a large peak of viral RNA (zone d) sedimenting between light ribosomal RNA and heavy chloroplastic ribosomal RNA. Zone a, 4-5 S RNA; b, DNA; c, light ribosomal RNA; d, 21 S RNA; e, heavy chloroplastic ribosomal RNA; f, heavy cytoplasmic ribosomal RNA.

Nucleic acids extracted from healthy barley or corn tissues (Fig. 2-A) consisted of transfer and 5 S RNA, DNA, light ribosomal RNA of chloroplastic and cytoplasmic origin, 21 S RNA of apparent chloroplastic origin, heavy ribosomal RNA from chloroplasts, and heavy ribosomal RNA from cytoplasm. Under these conditions, light ribosomal RNA from chloroplastic ribosomes was usually not resolved from that of cytoplasmic ribosomes. Viral RNA (21 S) was easily detected in extracts of infected leaves (Fig. 2-B) by the increase in the optical-density zone. Black Hulless barley seedlings became infected when inoculated with 21 S RNA from this gradient. Integration of the optical-density scanning patterns and correction for the amount of 21 S RNA in healthy tissue were completed for the inoculated and systemically invaded corn tissue and for the systemically invaded barley tissue (Table 1). Even though there was very little symptomatic evidence of BSMV infection in the inoculated corn leaves, an optical-density zone corresponding to viral RNA was detectable by three DPI. The quantity of viral RNA increased steadily until at least seven DPI. Extractions made from systemically infected corn leaves and meristems at 13, 18, 23, 32, and 41 DPI consistently revealed a negligible amount of viral RNA. Systemically invaded corn tissue harvested at 7 DPI produced a viral RNA yield (ca. 150 μg/g tissue) that was nearly comparable to that isolated from the highly susceptible Black Hulless barley (9). Intact viral nucleoprotein production in the corn tissues, however, was consistently low during the first wk after inoculation, and remained very low (10-75 μg/g) in inoculated tissue and in a variety of systemically invaded tissues harvested throughout the growth period of the plants.

Systemic invasion of leaf two of the BSMV-resistant Moreval barley was characterized by a longer incubation period and resulted in a much reduced viral RNA yield in comparison to the reaction of Black Hulless barley in similar experiments (9). Comparisons of the apparent viral RNA content in systemically invaded leaves of Moreval barley and the corn line indicated a lower viral RNA production in the former host (Table 1). Intact nucleoprotein production in Moreval, however, exceeded that of the corn variety.

BSMV-RNA and BSMV-RNA Rf in corn meristems.—Male line corn plants were grown at 20 C as previously described and were inoculated at 17 days after planting. At 13, 21, 35, and 49 days after inoculation, tissues of the tassel meristematic areas or tassel meristems were

excised under a binocular microscope and placed in 500 μCi of ^{32}P for 18 hr. The very small (ca. 1-mm diam) dome-shaped tassel meristems, harvested at 30 days after planting, probably included some extraneous tissue.

All excised tissues incorporated substantial quantities of ^{32}P into nucleic acids. Seven tassel meristem areas from the first sampling date yielded more than 500 μg nucleic acid, which was more than adequate for analysis on linear-log gradients. A subsample of each preparation was also used to prepare a crude RF fraction (10). Analysis of the preparations revealed that substantial quantities of incorporated ^{32}P was associated with 21 S single-stranded viral RNA at 13, 21, and 35 DPI, even though little increase in optical density of the 21 S zone was apparent. Data yielded by the earliest sample (13 DPI, Fig. 3-A) indicated that ^{32}P was being rapidly incorporated into viral RNA in meristematic tissue, with a very small 21 S zone present. Samples harvested for in vivo labeling at 35 DPI (Fig. 3-B) also showed a substantial rate of 21 S labeling in the presence of a very small optical-density zone. The small zone sedimenting between the 21 S viral RNA and heavy ribosomal RNA in Figs. 3-A and 3-B is presumably of proplastid or mitochondrial origin. Corn roots or pollen from healthy plants, extracted as previously described, also produced a small RNA zone in the 23-24 S region. Meristem preparations analyzed for RF presence showed a small amount of ^{32}P, resistant to 0.1 μg ribonuclease per ml or to 50 μg deoxyribonuclease per ml in 0.001 M $MgCl_2$, in tissue collected at 13, 21, and 35 DPI. A typical RF zone (Fig. 3-C, 21 DPI) sedimented at 12-13 S, which corresponds to the S value previously described (10).

Meristem samples collected at 49 DPI (66 days after planting) showed no evidence of RF, although a small increase of ^{32}P was associated with the 21 S viral RNA zone. Tassels from plants with and without systemic symptoms were also used for in vivo labeling. The main tassel branch was detached when the midportion of the tassel was exserting anthers. The bottom portion of the tassel, which had not exserted its anthers, was used for labeling. Substantial ^{32}P incorporation into RNA and DNA was detected, but no increase in optical density or radioactivity was associated with the 21 S region. Apparently viral RNA synthesis occurred in the tassel meristematic areas before the period of rapid differentiation, elongation, and ultimate emergence of the tassel. Attempts to detect intact viral nucleoprotein in the meristems were inconclusive; a virus zone was apparent in one experiment at 34 days after inoculation, but not at 26, 40, or 51 days.

Attempted detection of viral RNA in progeny.— In vivo ^{32}P-labeling has detected BSMV-RNA synthesis as early as 6-24 hr postinoculation (9). The sensitivity of the procedure suggested that it might be useful in the detection of alien RNA in progeny showing AR. Seed was collected from selfed, ND18-BSMV-infected male-line plants, and from a cross involving a BSMV-infected male-line plant as pollen donor and healthy male-line plants as maternal parent. Other lines used in this experiment included AR lines induced by infection of the original pollen parent with the Argentina mild strain of BSMV. These lines included those showing increased ratios of Pr (purple seed) over pr (red seed), and two "mosaic mimic" lines, which show a foliar mosaic. Plants of the "mosaic mimic" line show low seedling vigor, and a large percentage of the plants fail to survive longer than a few wk after planting. Seed of an AR line from wheat streak mosaic virus-infected pollen donors, received from G. F. Sprague, was also included in the experiments.

Leaves were detached and placed in water with 500 μCi of ^{32}P for 18 hr. The nucleic acids were then extracted and analyzed on linear-log gradients. No unusual optical-density or radioactivity patterns were apparent in any of the samples; Fig. 4 includes optical density-radioactivity profiles from the progeny of selfed, infected male-line plants (Fig. 4-A) and from the "mosaic mimic" line (Fig. 4-B). Soluble RNA, DNA, and three of the four ribosomal RNA species were present in usual quantities and were highly labeled. No evidence of the presence of BSMV-RNA RF was found.

In an attempt to detect infectivity from the AR progeny, nucleic acids were extracted from two lines. Part of the preparation was used to analyze the quality of the nucleic acids, and the remainder was used to inoculate Black Hulless barley seedlings. Concentrations of nucleic acid were 1.0, 0.1, and 0.01 mg/ml. The buffer was 0.035 M K_2HPO_4, 0.050 M glycine, pH 9.2, and bentonite was added at a concentration to equal the concentration of nucleic acids (μg/μg). Celite was used as an abrasive. No inoculated Black Hulless seedlings showed symptoms of BSMV infection. Unpublished experiments with free BSMV-RNA extracted from infected barley, indicate that infectivity would be detected at a viral RNA concentration of less than 1 μg/ml.

DISCUSSION.— Comparative assays of viral RNA and nucleoprotein production in the BSMV-infected male corn line and in Moreval barley suggest possible differential characteristics of the host reactions during the first wk after inoculation. The BSMV-resistant Moreval barley expressed mild symptoms of systemic invasion and was further characterized by a reduced synthesis of viral RNA. Severe symptoms developed in systemically invaded corn tissue, and viral RNA reached high levels in comparison to Moreval barley. Intact nucleoprotein production by the hosts, however, appeared to be inversely correlated with viral RNA yield. The data suggest that the corn variety was thus deficient in either the synthesis of viral coat protein or in assembly mechanisms. Host reactions resulting in "resistance" to virus infection may operate at the virus transcriptional or translational levels, or at the assembly level. Comparison of the Moreval barley data with that from similar experiments with the highly susceptible Black Hulless barley variety (9) indicate that the resistance of Moreval is certainly associated with a reduced capacity to support viral RNA synthesis. In those experiments, infection of Black Hulless resulted in a viral RNA production of up to 200 μg/g tissue, or about a 4-fold increase over the amount produced by the resistant Moreval variety. Variation at the assembly level has also been shown; increased temp resulted in an enhanced synthesis of tobacco mosaic virus RNA and of coat protein without an increase in the production of intact particles (6). Serological studies have shown early appearance of viral antigen for BSMV (4) and other viruses (7, 11), and recent reports (13, 18) indicate that viral coat protein production in infected tissues can be effectively monitored by acrylamide gel electrophoresis. The integration of these approaches in examining the reaction of "resistant" and "susceptible" hosts to

TABLE 1. Barley stripe mosaic virus nucleoprotein (μg/g tissue) and viral RNA yield (μg/g tissue) in systemically invaded leaf two of 'Moreval' (C.I. 5724) barley and in inoculated and systemically invaded "male-line" corn leaves 2-7 days after inoculation

DPI[a]	Inoculated corn[b]		Systemic corn		Systemic barley	
	Virus	RNA	Virus	RNA	Virus	RNA
2	0	0	—	—	0	0
3	4	7	—	—	0	0
4	11	34	—	—	14	0
5	19	64	0	21	78	2
6	118	83	68	112	151	12
7	122	99	120	153	214	51

[a] Days postinoculation.
[b] Viral RNA and nucleoprotein estimated by planimetry and integration of photometric scanning patterns from density-gradient centrifugation (9).

a virus would seem to be relevant in the interpretation of the nature of cellular response to viral pathogens.

The establishment of BSMV infection in the male corn line was characterized by an early invasion of the tassel meristem. It is striking that ^{32}P incorporation into BSMV-RNA in the meristem proceeded so rapidly without accumulation of the synthesized product. The specific activity of in vivo ^{32}P-labeled viral RNA undoubtedly exceeded that of host ribosomal RNA at 35 DPI, 22 days after viral RNA synthesis was first detected. Thus the meristem was capable of supporting rapid viral RNA synthesis for an extended period. Since it was difficult to detect intact virus in the meristems, it would appear that the degradation of viral RNA must have proceeded as rapidly as synthesis during this period, and that encapsidation mechanisms were largely inefficient. The capacity of the meristems to support viral RNA synthesis finally declined as the tassel differentiated and emerged. The early (13 DPI, 30 days after planting) detection of viral RNA suggests that viral invasion of the meristems preceded meiosis. Sprague and McKinney (15) suggested that the AR effect was initiated at or near meiosis, since plants without systemic symptoms did not produce the AR effect. Upper leaf and tassel meristems probably differentiate during the same period. Symptoms on upper leaves may indicate invasion of tassel meristems before differentiation, since BSMV spreads very slowly, if at all, in plants inoculated in the 8- to 10-leaf stage, or in plants inoculated at the 2- to 3-leaf stage and grown at 25 C or in a greenhouse.

No alien RNA species were detected in a variety of AR progeny by in vivo ^{32}P-labeling, followed by density-gradient analysis of the extracted nucleic acids. Two of these lines showed a definite phenotypic effect: the "mosaic mimic" line and a "Navajo mimic" line, the latter of which produces seed with a Navajo characteristic (16). The production of seed by the selfed, infected male line offered an opportunity to examine plants with a susceptible genotype and with a potential for pollen or ovary transmission. The inability to detect viral RNA synthesis, symptoms, or infectivity in progeny of the selfed line provides additional evidence that the AR effect is initiated within the original infected pollen-donor plant, and that the nature of the transmitted entity does not share these characteristics of the inducing virus.

The basis of the AR pheonomena must reside in an interaction between the virus, or its nucleic acid, and the genetic apparatus of the host. Recently, very low molecular weight RNA species (less than 10^5 daltons) have been found to be associated with tobacco ringspot virus infection (12), and have been described as the infectious agent of

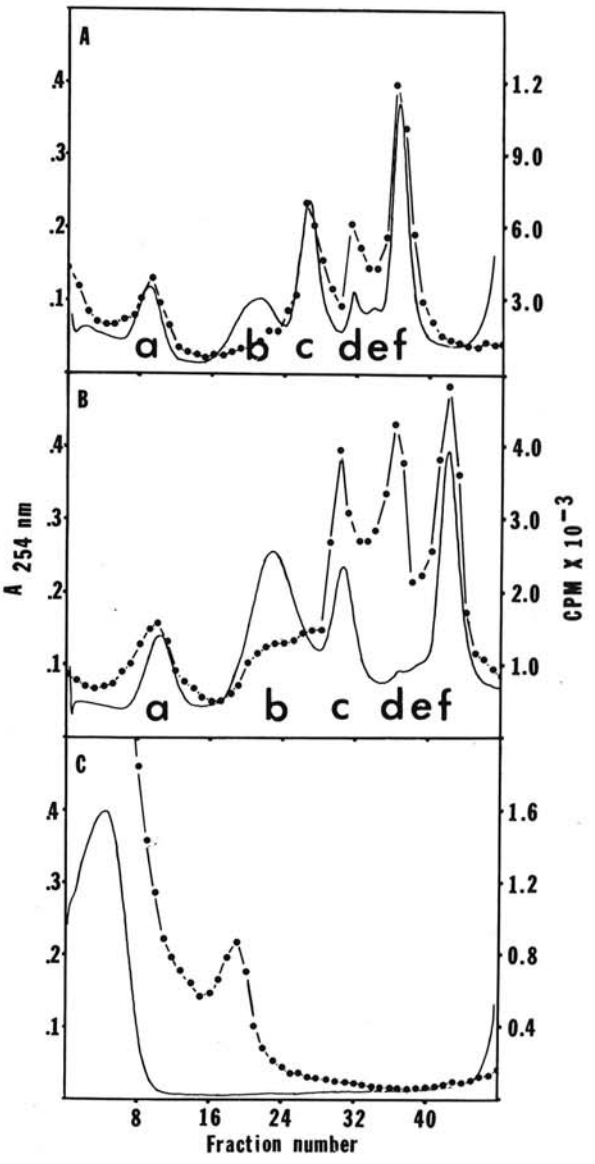

Fig. 3. Photometric scanning patterns and radioactivity (filled circles) of nucleic acids extracted from in vivo ^{32}P-labeled tassel meristems of barley stripe mosaic virus-infected male-line corn and subjected to density-gradient centrifugation on linear-log sucrose gradients in the Beckman SW 41 rotor. **A)** Nucleic acids from tassel meristems excised at 13 days postinoculation (DPI). **B)** As (A), except excised at 35 DPI. **C)** Partly purified replicative form barley stripe mosaic virus RNA prepared from tassel meristems harvested at 21 DPI. Zones a-f as in Fig. 2.

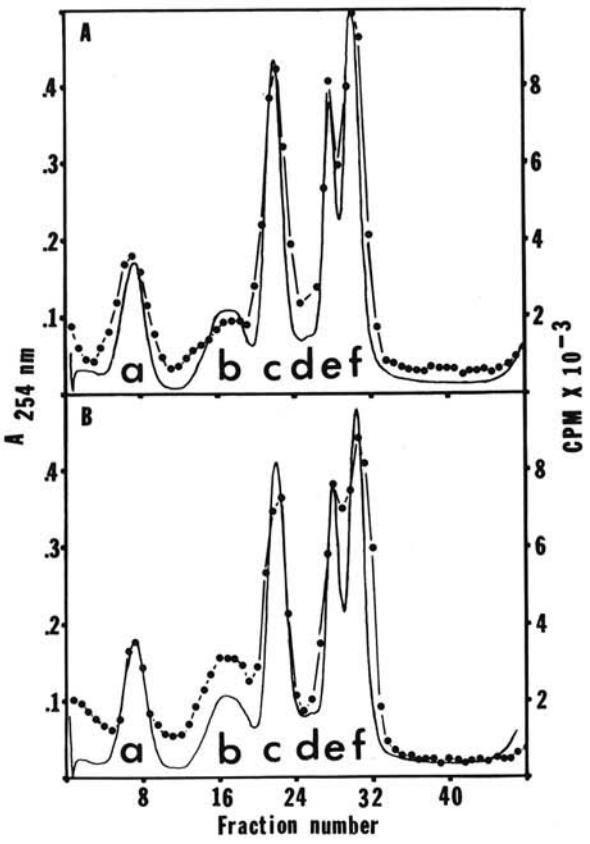

Fig. 4. Photometric scanning patterns and radioactivity (filled circles) of nucleic acids extracted from in vivo ^{32}P-labeled corn and subjected to density-gradient centrifugation on linear-log sucrose-density gradients in the Beckman SW 41 rotor. **A)** Nucleic acids extracted from progeny of selfed, infected male-line corn plants. **B)** Nucleic acids extracted from an "aberrant ratio" (AR) line showing a foliar mosaic ("mosaic mimic"). Zones a-f as in Fig. 2.

potato spindle tuber virus (PSTV) (3, 14). The low molecular weight of PSTV-RNA led Diener (3) to propose that the nucleic acid may be an abnormal regulatory RNA. Whether or not a similar virus-related or virus-induced agent is present in AR progeny is not discernible from these experiments. The observation that AR is not entirely stable may suggest that a viral-related nucleic acid, capable of multiplication in host resistant to multiplication of the entire viral genome, could possibly be related to AR. It has recently been established that BSMV may possess two (10) or possibly several (5) RNA species. It is relevant in this regard, however, to point out that, in addition to BSMV, wheat streak mosaic virus and lily fleck corn virus each can induce the AR effect, whereas experiments with strain "B" of sugarcane mosaic virus were negative (16). An interpretation of this most puzzling phenomenon must therefore include consideration of these virus-specific effects.

LITERATURE CITED

1. BRAKKE, M. K., and N. VAN PELT. 1970. Linear-log gradients for estimating sedimentation coefficients of plant viruses and nucleic acids. Anal. Biochem. 38:56-64.
2. BRAKKE, M. K., and N. VAN PELT. 1970. Properties of infectious ribonucleic acid from wheat streak mosaic virus. Virology 42:699-706.
3. DIENER, T. O. 1971. Potato spindle tuber "virus." IV. A replicating, low molecular weight RNA. Virology 45:411-428.
4. HAMILTON, R. I., and E. M. BALL. 1966. Antigenic analysis of extracts from barley infected with barley stripe mosaic virus. Virology 30:661-672.
5. JACKSON, A. O., L. C. LANE, and M. K. BRAKKE. 1972. Characterization of multiple RNA components from purified barley stripe mosaic virus. Phytopathology 62:767 (Abstr.).
6. KAJITA, S., and C. MATSUI. 1972. Effect of temperature on the synthesis of tobacco mosaic virus and X-protein in Nicotiana tabacum "Xanthi nc". Phytopathology 62:615-620.
7. LANGENBERG, W. G., and D. E. SCHLEGAL. 1969. Localization of tobacco mosaic virus protein in tobacco leaf cells during the early stages of infection. Virology 37:86-93.
8. MC KINNEY, H. H., and L. W. GREELEY. 1965. Biological characteristics of barley stripe mosaic virus strains and their evolution. Agricultural Research Service, U.S. Department of Agriculture Tech. Bull. 1324. 84 p.
9. PRING, D. R. 1971. Viral and host RNA synthesis in BSMV infected barley. Virology 44:54-66.
10. PRING, D. R. 1972. Barley stripe mosaic virus replicative form RNA: preparation and characterization. Virology 48:22-29.
11. SCHLEGAL, D. E., and D. E. DELISLE. 1971. Viral protein in early stages of clover yellow mosaic virus infection of Vicia faba. Virology 45:745-747.
12. SCHNEIDER, I. R. 1971. Characteristics of a satellite-like virus of tobacco ringspot virus. Virology 45:108-122.
13. SINGER, B. 1972. Protein synthesis in virus-infected plants II. The synthesis and accumulation of TMV and coat protein in subcellular fractions of TMV-infected tobacco. Virology 47:397-404.
14. SINGH, R. P., and M. C. CLARK. 1971. Infectious, low-molecular weight ribonucleic acid from tomato. Biochem. Biophys. Res. Comm. 44:1077-1088.
15. SPRAGUE, G. F., and H. H. MC KINNEY. 1966. Aberrant ratio: an anomaly in maize associated with virus infection. Genetics 54:1287-1296.
16. SPRAGUE, G. F., and H. H. MC KINNEY. 1971. Further evidence on the genetic behavior of AR in maize. Genetics 67:533-542.
17. SPRAGUE, G. F., H. H. MC KINNEY, and L. W. GREELEY. 1963. Virus as a mutagenic agent in maize. Science 141:1052-1053.
18. ZAITLIN, M. and V. HARIHARASUBRAMANIAN. 1972. A gel electrophoretic analysis of proteins from plants infected with tobacco mosaic and potato spindle tuber viruses. Virology 47:296-305.