Reversible Host Adaptation in Cucumber Mosaic Virus

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ABSTRACT

Occasional systemic infections of cucumber mosaic virus (CMV) occurred in cowpea when over 200 primary local lesions/plant developed on inoculated primary leaves, or when the inoculated leaves were heated. Most of these mutants in cowpea were different from each other, as indicated by differences in local lesions, systemic symptoms, and injury to cowpea; by degree of injury to cucumber; and by local lesions and incidence of systemic infection in bean. Mutants were usually less injurious to cucumber and always more injurious to cowpea than the parent culture. When these mutants were passed through cucumber they usually became less injurious to cowpea, and in 21 cases they returned to the type form of CMV that produced local lesions but did not go systemic or cause injury in cowpea. When these mutants were passed through cowpea they became more injurious to cowpea. CMV was more injurious to cucumber in the winter months, or in plants subjected to reduced light in summer, than to plants with summer daylight. Phytopathology 60:1117-1119.

Changes in viruses induced by host passage occur with bacterial (1), plant (3, 8), and animal (5) viruses. One of the best-studied examples with plant viruses, and one of the few cases of reversible change, is tobacco mosaic virus in leguminous plants (3). This reversibility has not been adequately confirmed. The present study with CMV constitutes a confirmation in principle, and may be a more dramatic and more easily manipulated example. CMV has been shown by Price (8) and others to be highly mutable, and Price further showed that mutants could be induced by passage through cowpea, although he later (8) interpreted these as mutants selected by, rather than induced by, the host. The additional contribution here is that mutants are shown to be associated with heavy infection and heating of the cowpea leaves, that host injury is shown to be affected by host passage, and that some of the mutants have been shown to return to the original type as a result of passage through cucumber.

MATERIALS AND METHODS.—The stock culture of CMV used in this study was isolated from *Delphinium ajacis* L. and maintained in cucumber (*Cucumis sativus* L. 'National Pickling' or 'Ashley'). Symptoms in cucumber, tobacco (*Nicotiana tabacum* L.), and sugarbeet (*Beta vulgaris* L.), and the production of small necrotic local lesions in cowpea (*Vigna sinensis* [Torner] Savi 'Blackeye') seemed typical of other strains of CMV over several years, when maintained in cucumber.

For purposes of this study, mutant is defined as a strain of CMV which became systemic in cowpea and did not produce the small necrotic local lesions on cowpea that are so characteristic of CMV. An original mutant (P) is one which appeared in a cowpea plant inoculated with stock CMV. Other mutant inoculum is identified in terms of the number of passages in cowpea (P) or cucumber (C) to which it had been subjected. In this terminology, PCCC would refer to a mutant which had been passed through cucumber three times in succession since its original appearance in cowpea.

Mutants were maintained for a maximum of four passages in cowpea and/or four passages in cucumber, then discarded. Then another group (usually four) was isolated and studied.

Plants were grown in 3-inch pots of a sand:peat:fertilizer mixture in the greenhouse. They were mechanically inoculated at about 10 days after seeding.

The stock culture in cucumber and the mutants in cucumber and/or cowpea were simultaneously transferred to healthy cucumber and cowpea, and noninoculated controls were maintained. The green wt of the new growth that formed after inoculation was measured at about 3 weeks after inoculation.

No other strain of CMV and no virus resembling CMV was maintained in the greenhouse during this study.

RESULTS.—*Production of mutants.*—Of some 36,000 (estimated) cowpea plants inoculated with stock CMV since the phenomenon of mutation was first observed in this study, only 98 were observed to develop systemic symptoms, though more plants might well have developed systemic symptoms if they had been held longer. Systemic infection on unheated plants resulted only in plants with 214 or more local lesions on the inoculated leaves, and most plants with 214 or more lesions did not show systemic infection. The average number of lesions on the 54 unheated plants that became systemic was 1,240. Of 158 cowpeas inoculated with stock CMV and heated 5-80 sec at 50 C or 1-15 sec at 55 C, at about 1 day after inoculation, 44 became systemic and the minimum number of lesions on the heated plants that became systemic was 90. In one trial of 24 plants, all were heated and none became systemic; and in one trial of 24 plants, all were heated and all became systemic. It is therefore clear that while heating may favor systemic infection, it is very unreliable with present knowledge. The mutants produced in heated plants did not appear to differ as a group from the mutants resulting from heavy infection only.

Each mutant appeared to differ from each other mutant in that if four original mutants were transferred to three cowpeas each, the systemic symptoms in each group of three cowpeas were similar, but symptoms in the four groups were distinct from each other.

Comparative injury to cucumber and cowpea.—Mu-

tants were usually distinctive from each other in cowpea, but much less so in cucumber. Systemic symptoms in cucumber were a mild to severe mosaic, with the green wt of the new growth beyond the primary leaves amounting to from 0.12 to 1.26 times that of the noninoculated controls (Table 1) in 134 trials. This quantitative injury to cucumber depended in part at least on light and/or season. The average green wt of the infected cucumbers as a fraction of the green wt of the controls for the different months of the year, with the number of trials following the decimal valve, was as follows: January 0.18, 3; February 0.24, 2; March 0.32, 4; April 0.39, 5; May 0.51, 4; June 0.47, 2; July 0.30, 1; August 0.26, 2; October 0.28, 3; November 0.25, 3; and December 0.28, 6. The greatest injury was clearly in the darkest month. As a further test of the light effect, inoculated and control plants were held in darkness for different periods starting several days after inoculation. The green wt of the inoculated plants as a decimal fraction of the green wt of the controls (average of three trials, one inoculated on 13 June and dark treatments started 20 June, one inoculated 23 June and dark treatments started 23 July, and one inoculated 29 June and dark treatments started 8 July) was 0.60 for no dark treatment, 0.58 for 1 day in dark, 0.42 for 2 days in dark, 0.33 for 3 days in dark, and 0.22 for 4 days in dark. In one trial, all inoculated plants were killed by 3 days in dark. In another almost unrelated study, it was shown that healthy and CMV-infected cucumbers were more injured by exposure to darkness than were healthy or CMV-infected beans or cowpeas.

Symptoms in cowpea ranged from an ordinary mosaic to vein-clearing to no clear symptoms, with the green wt of the growth beyond the inoculated primary leaves ranging from 0 to 1.11 times that of the controls. The complete or 99% suppression or killing of the new growth of cowpea as a result of infection with CMV mutants occurred in three trials and with two different mutants, but was never an initial manifestation of a mutant.

Response of bean.—Though there are many reports of CMV in bean, bean is usually regarded as resistant to CMV. The present stock culture is considered typical in that it normally produced necrotic lesions about 0.1 mm in diam in the primary leaves of Pinto bean. The mutants of this study, on the other hand, produced lesions up to 12 mm in diam in bean, and some induced systemic symptoms. As with cowpea, each original mutant transferred to bean appeared different from every other, but the lesions from a single mutant were usually uniform. Bean appeared to be an excellent host for detecting differences in mutants produced in cowpea, but original mutants were never recognized to form in bean.

Return to normal type.—When mutant CMV was transferred from systemic cowpea to healthy cowpea, local lesions typical of ordinary CMV never appeared. Such transfers commonly resulted in large, faint chlorotic lesions with no necrosis; sometimes the large lesions were partly necrotic, and sometimes no lesions were observed, though the cowpeas typically developed systemic symptoms.

When mutant CMV was transferred from cucumber to cowpea, chlorotic lesions on the inoculated leaves followed by systemic infection typically resulted, but the lesions were more commonly necrotic than in the transfer of mutants from cowpea to cowpea. In several transfers of mutants from cucumber to cowpea, two types of lesions, one large chlorotic and one small necrotic, appeared. These cases probably represent a partial change in cucumber from the mutant to typical CMV. All such plants became systemically infected. In 21 transfers of mutants from cucumber to cowpea, local lesions typical of type CMV, and only such, appeared. Of these 21 cases, three appeared after only one passage through cucumber, 11 appeared after two passages, three after three passages, and four after four passages. These 21 cases are believed to represent a complete cycle from ordinary CMV to mutant CMV and back to typical CMV, though not necessarily exactly the same as the original. In all these 21 cases,

TABLE 1. Effect of host passage of CMV on injury to cucumber and cowpea

	Inoculation to cucumber			Inoculation to cowpea		
		Green wt[a]			Green wt[a]	
Inoculum	Trials	Range	Avg	Trials	Range	Avg
Regular CMV in cucumber	38	0.033-0.74	0.29	32	0.63-1.35	1.02
Initial mutant in cowpea	42	0.23-1.1	0.60	46	0.13-1.1	0.40
2 passages in cowpea	12	0.14-0.95	0.55	12	0.01-0.62	0.33
3 passages in cowpea	6	0.17-0.81	0.43	4	0.05-0.78	0.33
4 passages in cowpea	2	0.18-0.9	0.54	2	0.0-0.34	0.17
Mutant passed						
1 × through cucumber	20	0.1-0.58	0.29	22	0.035-0.98	0.38
2 × through cucumber	4	0.39-0.85	0.55	4	0.0-0.83	0.41
3 × through cucumber	2	0.23-0.46	0.34	2	0.39-1.11	0.83
All mutant passages ending in cucumber	42	0.16-0.92	0.36	92	0.0-1.11	0.47
All mutant passages ending in cowpea	92	0.12-1.26	0.53	91	0.0-0.78	0.35

[a] Expressed as $\frac{\text{green wt of new growth of inoculated plants}}{\text{green wt of new growth of control plants}}$. The green wt of the new growth of control cucumbers ranged from 4 to 77 g and averaged 21 g. The green wt of the new growth of the control cowpeas ranged from 4 to 44 g and averaged 15 g.

the mutants were more injurious to cucumber than the normal mutants in cowpea.

Tests were not carried beyond four passages of the mutants through cucumber, but with four passages through cucumber, most mutants retained the property of giving systemic infection in cowpea.

DISCUSSION.—That these mutants are strains of CMV and not contaminants is indicated because (i) they produced typical symptoms of CMV in cucumber; (ii) they did not resemble any other virus under study; (iii) systemic infections in cowpea were resistant to but not immune to stock CMV; (iv) they occurred frequently and most of them were different from each other; (v) some of them returned to typical CMV after passage through cucumber; and (vi) precautions had been taken to avoid contamination.

That these mutants were not present in the stock culture is indicated by the much later appearance of symptoms of original mutants in cowpea than from the transfer of mutants from cowpea to cowpea, even with very dilute inoculum, and by their failure to appear in several thousand cowpeas infected with stock CMV to give less than 200 lesions/plant.

The question as to whether these mutants were induced by the host or were selected by the host from random mutations cannot be settled from the evidence presented, and the literature is not satisfying. Price at one time (8) apparently believed his CMV mutants were induced by host passage, but later (9) apparently believed that they were random mutants selected by the environment. Similarly, Bawden (4) believes that mutants are random and selected by the host, while Mundry (7) believes that they can be induced by the substrate.

The most definitive work on adaptation of microorganisms to a substrate is with acquired tolerance of bacteria to antibiotics. Dean & Hinshelwood (6) and Barber (2) indicate that adaptation is induced by the substrate, but Stanier et al. (10) favor the idea that they are merely selected by the substrate.

I believe that induction by the substrate is the most likely explanation of the mutants observed here.

LITERATURE CITED

1. ARBER, W. 1965. Host controlled modification of bacteriophage. Annu. Rev. Microbiol. 19:365-380.
2. BARBER, M. 1953. Antibiotic-resistant staphylococcal variants, p. 235-252. *In* R. Davies & E. F. Gale [ed.] Adaptation in microorganisms. Cambridge Univ. Press.
3. BAWDEN, F. C. 1958. Reversible changes in strains of tobacco mosaic virus from leguminous plants. J. Gen. Microbiol. 18:751-766.
4. BAWDEN, F. C. 1964. Plant viruses and virus diseases. Ronald Press, N. Y. 361 p.
5. BRIODY, B. A., W. A. CASSEL, J. LYTH, & M. FEARING. 1933. Adaptation of influenza virus to mice. Yale J. Biol. Med. 25:391-400.
6. DEAN, A. R. C., & C. HINSHELWOOD. 1953. Observations on bacterial adaptation, p. 21-45. *In* R. Davies & F. F. Gale [ed.] Adaptation in microorganisms. Cambridge Univ. Press.
7. MUNDRY, K. W. 1959. The effect of nitrous acid in tobacco mosaic virus: mutation, not selection. Virology 9:722-726.
8. PRICE, W. C. 1934. Isolation and study of some yellow strains of cucumber mosaic. Phytopathology 24:743-761.
9. PRICE, W. C. 1964. Strains, mutation, acquired immunity and interference, p. 93-117. *In* M. K. Corbett & H. D. Sisler [ed.] Plant Virology, Univ. Florida Press, Gainesville.
10. STANIER, R. Y., M. DOUDOROFF, & A. E. ADELBERG. 1963. The microbial world. Prentice-Hall, Englewood Cliffs. 753 p.