Symptom Variation in Different *Arabidopsis thaliana* Ecotypes Produced by Cauliflower Mosaic Virus

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ABSTRACT

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To determine the role of host factors in the systemic infection of *Arabidopsis thaliana* by cauliflower mosaic virus (CaMV), variation in systemic symptoms produced by three different viral isolates in 23 different *A. thaliana* ecotypes was studied. Differences in the overall pattern of viral symptoms were observed when different *A. thaliana* ecotypes were inoculated with a given CaMV isolate. In most cases, the pattern differences correlated with the rate of plant development. Symptoms on early flowering ecotypes were confined to the uppermost parts of the plant, the flower stalk and cauline leaves, whereas symptoms on late flowering ecotypes were more widespread, appearing on the rosette leaves. One ecotype, En-2, appeared to be resistant to CaMV for reasons unrelated to plant development. CaMV appears to replicate and move from cell to cell in the inoculated leaves of this ecotype, but it does not move systemically. Differences were also observed in the ability of different viral isolates to produce symptoms in a given *A. thaliana* ecotype. In general, one viral isolate, W260, produced more severe symptoms than the other two isolates, CM4-184 and CM1841.

Additional keywords: long-distance virus movement, natural variation, systemic virus movement, virus resistance.

Natural variation among different cultivars of a crop species has been an important source of resistance genes to plant viruses (3). In a classic study on the genetic resistance of *Nicotiana glutinosa* to tobacco mosaic virus (TMV), Holmes described sources of virus resistance in different cultivars of crops and related wild varieties (5). Since then, in a number of cases, the genetic basis for virus resistance was found to be controlled by single Mendelian determinants (3). For example, the *Tm-1* gene of tomato blocks the replication of TMV in tomato, and the *Tm-2* gene inhibits cell-to-cell spread of TMV in tomato (13,17). Kuhn has identified a single dominant gene in cowpea that inhibits the systemic spread of cowpea chlorotic mottle virus (8). It has also been demonstrated that single viral genes can determine virulence and overcome host resistance (13,16).

Although several of the above-mentioned plant resistance genes have been agronomically important and led to the production of more resistant cultivars, the molecular mechanisms underlying these resistances are not known. There is renewed interest in exploring variation in virus resistance and symptom formation in *Arabidopsis thaliana* (L.) Heynh. because the genetics and molecular biology of this cruciferous plant make it possible to identify and clone genes responsible for various phenotypes (14). To date, examples of variation found in *A. thaliana* include a mutant that produces reduced levels of TMV coat protein (7). Ecotype variation in virus resistance has also been observed in *A. thaliana*. The Dijon ecotype of *A. thaliana* has been reported to be more resistant to turnip crinkle virus than the Col-0 ecotype (10). Balazs and Lebeurier have screened different ecotypes of *A. thaliana* for their susceptibility to cauliflower mosaic virus (CaMV) infection and have reported differences (1).

There has also been much interest in finding variation among CaMV isolates that infect *A. thaliana* or related hosts. For example, Daubert et al tested the ability of several CaMV isolates to infect different cruciferous and solanaceous hosts (2). Likewise, Melcher examined the ability of various CaMV isolates to infect turnip and *A. thaliana* (11). Through the use of chimeric genomes constructed between different CaMV isolates, Schoelz et al found that the ability of CaMV to propagate and produce symptoms in several hosts could be attributed to the action of gene VI (16).

In an effort to determine whether host factors can influence CaMV systemic movement, the severity and distribution of visible viral symptoms on different *A. thaliana* ecotypes were determined. Variation in visible symptoms was observed between different isolates of CaMV on a given ecotype and between different ecotypes when inoculated with the same CaMV isolate. Much of the variability could be attributed to differences in the rate of plant development.

MATERIALS AND METHODS

Ecotypes used. The *A. thaliana* ecotypes used in this study were obtained from Robert Last at the Boyce Thompson Institute for Plant Research and from the Arabidopsis Information Service, Frankfurt, Germany. Seeds were planted in Redi Earth mix (W. R. Grace & Co., Cambridge, MA) and vernalized for 2 wk at 4 C in fiber pots, 4 in. in diameter, covered with Saran Wrap. The plants were then placed in a light room under continuous illumination at 60 μE m^{-2} s^{-1} of photosynthetically active radiation at (PAR) 21 C. Three days after the seeds germinated, the plastic wrap was removed. Plants were subirrigated by soaking pots in a plastic flat filled with water for 3–4 h. After 16 days, the plants developed four to six true rosette leaves and were thinned to 20 plants per pot. Plants were then inoculated with virus on the day after thinning.

Viral isolates, inoculation, and growth conditions. CaMV isolates CM4-184, CM1841, and W260 were maintained by serial passage in *Brassica campestris* var. *rapa* L. 'Just Right' (turnips) in a greenhouse. For each viral isolate, 20 *A. thaliana* plants were mechanically inoculated with cell sap prepared by grinding infected turnip leaves in 10 mM potassium acetate buffer (pH 7.2) at 3 ml buffer per gram of tissue. Celite was added at 6 mg ml^{-1} of cell sap, and 5 μl of suspension was rubbed on three leaves per plant (leaves 2, 3, and 4) with a plastic spatula. Leaves were rinsed with water 5 min after inoculation, and inoculated plants were incubated for 8 h at room temperature under room lights. Plants were then grown in a growth chamber under a cycle of 12 h light and 12 h dark at 19 C. The total photosynthetic active radiation of about 60 PAR was provided by sodium and

mercury vapor lamps. Plants were rotated to different locations within the chamber every 2 days to assure even lighting. Plants were observed every day for systemic symptoms and finally assessed for symptoms at 41 days postinoculation.

Leaf skeleton hybridization. *A. thaliana* leaves were removed from plants and subjected to leaf skeleton hybridization as described by Melcher et al (12), except that the leaves were extracted initially with ethanol instead of 2-methoxyethanol and then treated with 0.5 M NaOH–1.0 M NaCl and 1.0 M Tris-HCl (pH 7.5)–1.5 M NaCl instead of 0.5 M NaOH–1.5 M NaCl and 0.5 M Tris-HCl (pH 7.0)–3.0 M NaCl following the proteinase K step. The 8-kb insert from the plasmid pLW414 (the cloned genome of the CM4-184 isolate of CaMV [6]) was labeled with a Multiprime DNA labeling kit (Amersham Corp., Arlington Heights, IL) according to manufacturer's instructions and used as a probe.

RESULTS

Variation in symptoms produced by different CaMV isolates. In general, CaMV causes systemic chlorotic lesions and veinclearing symptoms on rosette and cauline leaves, and mottling and chlorosis of flower stalks and siliques (seed pods) in *A. thaliana* (1,11). To examine the symptoms produced by different CaMV isolates, a single susceptible *A. thaliana* ecotype, Rsch-4, was inoculated with one of three different CaMV isolates. The three viral isolates, CaMV CM4-184 (6), CM1841 (4), and W260 (15), produced relatively mild, moderate, and severe symptoms, respectively, on turnip under our growth conditions. Severity of symptoms was based on the intensity of chlorosis, stunting, and the extent to which any affected organ showed symptoms. As in turnip, CaMV isolate W260 generally produced severe symptoms on *A. thaliana* ecotype Rsch-4 (Fig. 1D). In these plants, intense chlorosis and veinclearing was observed on rosette and cauline leaves, and the flower stalks were mottled and stunted. On the other hand, CaMV isolates CM4-184 and CM1841 generally produced milder symptoms on *A. thaliana*. Plants inoculated with CaMV CM4-184 had chlorotic symptoms on cauline leaves only, and the infected plants were mildly stunted (Fig. 1B). Plants inoculated with CaMV CM1841 had chlorotic lesions on the cauline leaves and the flower stalks, and the infected plants were moderately stunted (Fig. 1C).

Variation in symptoms produced on different *A. thaliana* ecotypes. Symptoms also varied when different *A. thaliana* ecotypes were inoculated with the same CaMV isolate. When three selected *A. thaliana* ecotypes were inoculated with CaMV isolate CM4-184, systemic symptoms ranged from no visible symptoms to severe stunting and extreme chlorosis (Fig. 2). The *A. thaliana* ecotype En-2 is an example of an ecotype in which inoculated plants showed no visible systemic symptoms (Fig. 2A). Ecotype Kas-1 showed moderate systemic symptoms. Veinclearing and chlorotic mottle symptoms in Kas-1 plants were limited to the cauline leaves and flower stalks, and the infected plants were slightly stunted (Fig. 2B). On the other hand, infected *A. thaliana* ecotype RLD plants had more severe, widespread, systemic symptoms. Veinclearing symptoms were visible on rosette leaves as well as cauline leaves, flower stalks were chlorotic, and the plants were severely stunted (Fig. 2C).

A broader survey of 23 different *A. thaliana* ecotypes inoculated with three different CaMV isolates was conducted (Fig. 3). A total of 60 plants of each ecotype was inoculated with the three different viral isolates (20 plants per viral isolate). It was found that certain ecotypes, such as Fr-2, Pla-1, and RLD, were highly susceptible to all three CaMV isolates and exhibited widespread systemic symptoms on rosette leaves, cauline leaves, and flower stalks. There were also instances where an ecotype was asymptomatic when inoculated with one or two isolates of CaMV but not with all three. For example, ecotype Tsu-0 showed no visible systemic symptoms when inoculated with either CM4-184 or CM1841, but it showed widespread, visible symptoms on rosette leaves, cauline leaves, and flower stalks when inoculated with

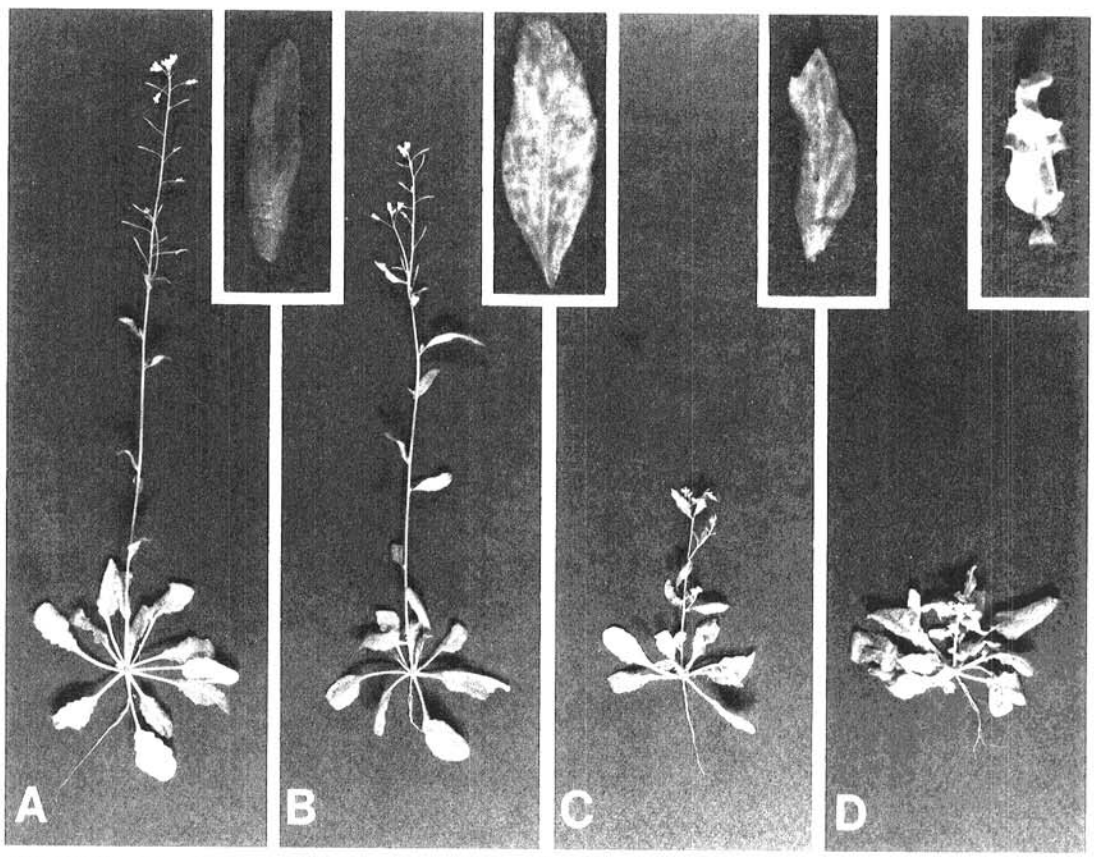

Fig. 1. Variation in symptoms produced by different CaMV isolates. *Arabidopsis thaliana* ecotype Rsch-4 was inoculated on the rosette leaves with one of three different CaMV isolates (CM4-184, CM1841, and W260) and then photographed at 41 days postinoculation. **A,** Uninoculated plant. **B,** Plant inoculated with CaMV CM4-184. **C,** Plant inoculated with CaMV CM1841. **D,** Plant inoculated with CaMV W260. Inset shows a representative cauline leaf from the plant to the left of inset.

W260. Likewise, ecotype Be-0 exhibited no visible systemic symptoms when inoculated with W260 but had limited systemic symptoms in flower stalks and cauline leaves when inoculated with either CM4-184 or CM1841.

An interesting pattern emerged when the *A. thaliana* ecotypes were rank-ordered with respect to rate of development or time to bolting (Fig. 3). In general, systemic symptoms were more widespread in the late flowering ecotypes such as RLD, described above. In ecotype RLD the flower stalks were chlorotic, and veinclearing was evident on both rosette and cauline leaves (Fig. 2C). On the other hand, symptoms were confined to the upper regions of the plant in the more early flowering ecotypes such as Kas-1 described above. In ecotype Kas-1, the flower stalks were chlorotic, but veinclearing symptoms were only observed on the cauline leaves (Fig. 2B).

Interesting exceptions to the general trend relating the pattern of symptoms to the time of bolting were also observed. Three *A. thaliana* ecotypes, En-2, Sv-0 and Wil-2, have very different rates of development but nonetheless were symptomless when inoculated with any one of the CaMV isolates tested. Both En-2 and Sv-0 developed more slowly than the standard *A. thaliana* ecotype Col-0, which has widespread, visible systemic symptoms, yet inoculated En-2 and Sv-0 plants appeared symptomless. To determine whether the lack of systemic symptoms in the En-2 ecotype was due to the absence of virus in leaves that would normally be invaded during systemic infection, the physical presence of the virus in both the inoculated and the youngest rosette leaves was determined with a leaf skeleton hybridization procedure (11,12). Sixty plants of the En-2 ecotype inoculated with CaMV isolate CM4-184 were examined in this way, and only a few representative leaves are shown. In turnip, CaMV systemically invades a predictable group of younger leaves from a given inoculated leaf (9). Therefore, by examining the youngest rosette leaves in inoculated plants of the En-2 ecotype, we could demonstrate by the presence of a hybridization signal whether the virus had systemically invaded the plant. In En-2 plants, virus appeared to accumulate locally in inoculated leaves (Fig. 4C), but we found no evidence for the spread of virus to other leaves (Fig. 4E and G); in the standard *A. thaliana* ecotype Col-0, however, both the inoculated leaf (Fig. 4B) and leaves targeted for systemic infection (Fig. 4D and F) showed a strong hybridization signal. Therefore, we conclude that *A. thaliana* plants of the En-2 ecotype are not simply refractory to virus infection, but are resistant to CaMV isolate CM4-184. Furthermore, the resistance to CaMV in the En-2 ecotype appears to manifest itself at the level of the systemic spread of the virus.

DISCUSSION

In this study, we found differences among *A. thaliana* ecotypes in the production of symptoms resulting from CaMV infection. In most cases, the extent or pattern of systemic symptoms appeared to be influenced by the rate of plant development. The general trend was that systemic symptoms were confined to the flower stalk and cauline leaves in early flowering ecotypes; but in late flowering ecotypes, symptoms were more widespread,

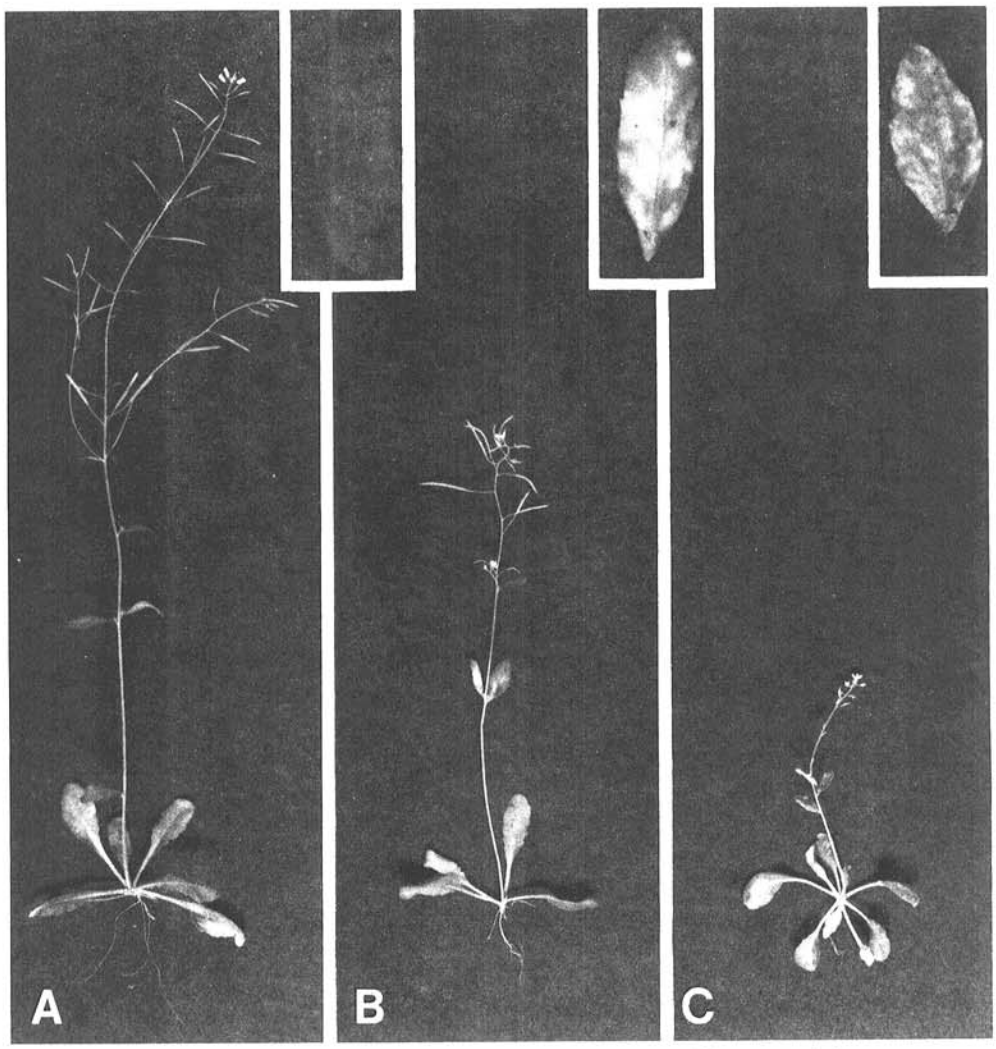

Fig. 2. Variation in symptoms produced on different *Arabidopsis thaliana* ecotypes. Three different *A. thaliana* ecotypes were inoculated on the rosette leaves with CaMV isolate CM4-184 and photographed at 41 days postinoculation. **A,** Ecotype En-2. **B,** Ecotype Kas-1. **C,** Ecotype RLD. Inset shows a representative cauline leaf from the plant to the left of the inset.

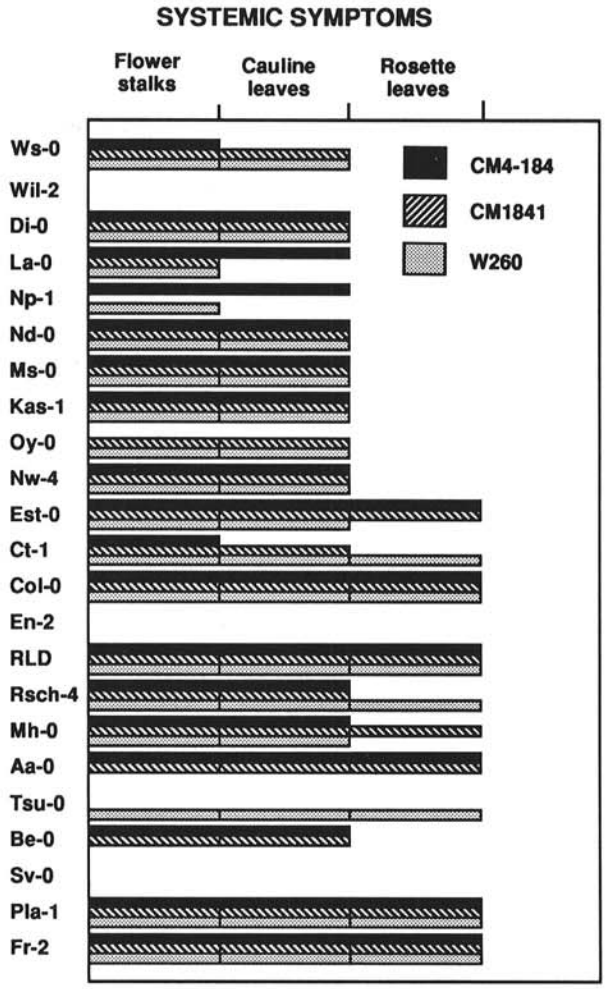

Fig. 3. The pattern of viral systemic symptoms varies with the rate of plant development (time to bolting). Twenty-three different *Arabidopsis thaliana* ecotypes were inoculated on the rosette leaves with three different viral isolates (CM4-184, CM1841, and W260) and then observed for systemic symptoms at 41 days postinoculation. The ecotypes are arranged in the order in which the plants bolted under the specified growth conditions (top to bottom, early to late flowering). Times to bolting (in days) are: Ws-0 = 15.5, Wil-2 = 16, Di-0 = 16.5, La-0 = 17, Np-1 = 17.5, Nd-0 = 18, Ms-0 = 18.5, Kas-1 = 19, Oy-0 = 20, Nw-4 = 20.5, Est-0 = 21, Ct-1 = 21.5, Col-0 = 22, En-2 = 27, RLD = 31, Rsch-4 = 32, Mh-0 = 33, Aa-0 = 38.5, Tsu-0 = 39, Be-0 = 39.5, Sv-0 = 40, Pla-1 = 40.5, and Fr-2 = 41 days. Bars indicate the presence of systemic symptoms in various organs.

appearing on the rosette leaves. In turnip, Melcher found a leaf-age dependence of symptom production, in that young leaves could be systemically infected with CaMV, but fully mature turnip leaves could not (11). We found in other studies in turnip that the leaf-age dependence was due to the fact that leaves can import virus for only a limited period of time during leaf development (9). The conclusions arrived at in these papers may explain the effect of plant development on virus distribution observed in this study.

However, what stands out against the general developmental trend are the exceptions—those ecotypes that are mid- to late-flowering but do not show symptoms. For example, *A. thaliana* ecotype En-2 does not produce symptoms in response to any one of the three viral isolates, and leaves into which the virus might potentially move show no evidence of virus as assessed by in situ hybridization techniques. However, CaMV does appear to replicate and move locally in the inoculated leaves suggesting that the plants are simply not refractory to mechanical inoculation but resist systemic CaMV movement. The fact that *A. thaliana* ecotype En-2 is resistant to the three CaMV isolates tested here is interesting because it is apparently not resistant to all CaMV

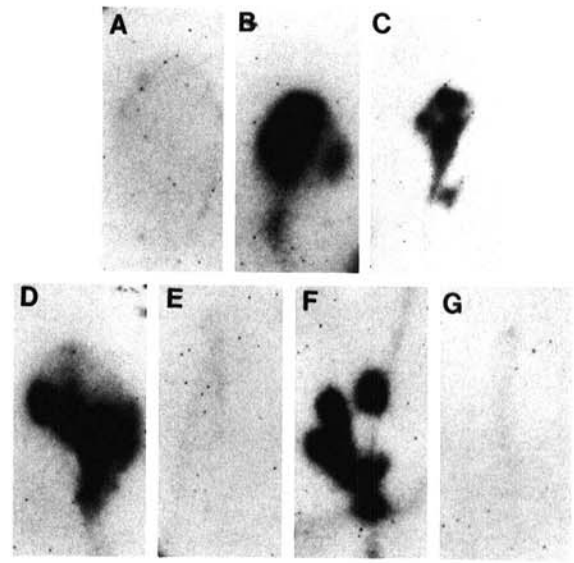

Fig. 4. *Arabidopsis thaliana* ecotype En-2 is resistant to CaMV. Leaves from Col-0 and En-2 ecotype plants were removed at 41 days postinoculation and subjected to leaf skeleton hybridization with a CaMV probe. Autoradiograms of representative leaves are shown. **A,** Uninoculated leaf; **B and C,** inoculated leaf; **D–G,** young rosette leaves. **B, D, F,** ecotype Col-0; **C, E, G,** ecotype En-2.

isolates. Balazs et al (1) reported that the En-2 ecotype was susceptible to the Cabb-S and D/H isolates of CaMV. It is also possible, however, that the En-2 plants used in their study were actually different plants because they were obtained from a different source.

Therefore, in the group of *Arabidopsis* ecotypes examined in this study, the pattern of symptoms was influenced in most cases by the rate of plant development and in a few cases by factors that were independent of the rate of plant development, such as in the En-2 ecotype plants. The host factors determining resistance to systemic CaMV movement in the En-2 ecotype might be complex, involving many genes, or it might be simple. We are in the process of determining the genetic basis for the resistance trait in this ecotype.

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