The Segregation of an Italian Virulent Isolate of Endothia parasitica Into H and V Types

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ABSTRACT.—The Italian virulent isolate EP-49, reported by Day et al. (1977) to possess dsRNA is capable of giving rise to hypovirulent types. When conidia were plated, all produced typical virulent colonies. However, if conidia were irradiated with UV light, up to 20 percent of the surviving colonies were hypovirulent. Both the virulent and hypovirulent colonies possessed dsRNA. Unstable virulent strains containing dsRNA such as EP-49, which can segregate into hypovirulent forms, are here termed suppressed hypovirulent(S) strains.

In the context of Endothia parasitica (Murr.) P. J. & H. W. And. research, the term hypovirulence has been reserved to denote the suppression of virulence by a cytoplasmically transmissible factor (Van Alfen et al., 1975; Grente and Sauret, 1969). The relative growth of cankers in chestnut trees has been the usual means of determining virulence, and without this as part of the definiation, the term is meaningless. Reduced canker growth alone, without its being cytoplasmically transmissible, is not hypovirulence. Likewise, cytoplasmic transmission of other characters, such as reduced sporulation, alone is not hypovirulence.

Working with a large number of hypovirulent (H) and virulent (V) strains obtained from Europe as well as the U.S., Elliston (1977; 1979) reported a continuum of virulent responses ranging from avirulence to normal virulence. All strains he tested having less than normal virulence, and some as virulent as normal strains, possessed double-stranded RNA (dsRNA). He termed these strains diseased (Elliston, 1979). However, Elliston implied (1977; 1979) and Day et al. (1977) stated that these strains are hypovirulent. This is a departure from previous definitions of hypovirulence. Some of the strains considered to be hypovirulent are as virulent as normal strains, and distinguishable from normal strains only on the basis of fewer pycnidia and presence of dsRNA. Day et al. (1977) reported that dsRNA was associated with hypovirulence in all strains he tested. However, this correlation was based upon Elliston's (1977; 1979) concept that some strains, though virulent in pathogenicity tests, are hypovirulent.

We do not question the validity of observations by Elliston (1977; 1979) and Day et al. (1977). In support of their observations our data indicate that the dsRNA containing strains which produce virulent cankers are unstable and give rise to hypovirulent strains. These virulent dsRNA containing strains must therefore possess the hypovirulent factor, but in a suppressed state. To call these suppressed strains hypovirulent, however, is confusing.

HYPOVIRULENT INSTABILITY

One of the characteristics many hypovirulent strains exhibit is instability. Hypovirulent strains frequently revert to normal virulence. Grente and Sauret (1969) previously reported that all hypovirulent strains are unstable, i.e., when grown from single conidia they do not necessarily produce the clonal type, but in fact may give rise to any of the other hypovirulent types or the virulent type. Grente (1979) now reports that the JR hypovirulent type (JR) is stable, giving rise only to other JR types. The Italian workers Bonifacio and Turchetti (1973) also recognized the existence of three different hypovirulent types including one stable pigmented type, P, which by their description corresponds to Grente's JR.

Although the instability evidently is not characteristic of all hypovirulent strains, it has proven to be a useful tool in understanding the nature of hypovirulence. The studies reported here are an attempt to elucidate some of the characteristics of the strains of E. parasitica possessing the hypovirulent factor.

In our studies of hypovirulence we have used primarily two strains, EP-11 and EP-49. EP-11 was derived by heterokaryosis between Grente's B hypovirulent strain and an American virulent strain. EP-49, obtained from Italy, is as virulent as a normal strain when tested for pathogenicity; yet it carries dsRNA. The French B strain has proven to be unstable in culture, readily generating both orange and white colonies when grown from single spores for 7-10 days under light. When tested for virulence in chestnut trees, the pigmented colonies were virulent, but the white colonies were able to initiate only small cankers before growth was arrested by host responses. The white colonies are thus typical of the hypovirulent type of growth in a tree. Over 100 colonies have been so tested for virulence, and to date there has been a complete correlation between color and virulence. Evidently JR types are not produced frequently since according to Grente and Sauret (1969) they are both pigmented and hypovirulent.

Using pigmentation as a basis for distinguishing between normal and hypovirulent types, we have found that hypovirulent strains are quite unstable. The age of the culture, the medium to which the spores are transferred, and irradiation by ultraviolet light (Van Alfen *et al.,* 1978) were all shown to affect stability.

EFFECTS OF IRRADIATION ON HYPOVIRULENCE

When irradiated with a dose of UV light sufficient to kill 90-99 percent of the conidia, virulent strains yielded very few white colonies. These colonies, when tested for virulence, proved to be avirulent. This pattern is typical of normal genetic mutations.

If hypovirulent strains are irradiated in the same manner (Van Alfen *et al.,* 1978) however, the irradiation induces a change in the ratio of pigmented to white colonies. In a typical experiment, before irradiation 90 percent of the conidia yielded white colonies, but after irradiation, 70 percent of the surviving colonies were pigmented. One explanation of this phenomenon might be that conidia yielding white colonies are much more sensitive to killing by UV irradiation than those yielding pigmented ones. It has been found, however, that hypovirulent colonies are less sensitive to UV irradiation than normal virulent ones. It has also been found that X-ray irradiation, at a dose sufficient to kill over 50 percent of the spores, had no effect on pigmentation of EP-11 colonies (Van Alfen *et al.,* 1978).

UNSTABLE VIRULENT STRAINS

In our experiments with hypovirulent strains of *E. parasitica* we observed that some of the virulent colonies derived from unstable hypovirulent strains were also unstable. This type of behavior of apparently normal strains has not been reported by others. The following is an example of the type of experiment that demonstrates the instability of some of these unusual virulent colonies. After plating conidia of EP-11, the pigmented colonies that arose were transferred by mycelial plug onto fresh potato dextrose agar (PDA). Seventy-eight percent reverted from pigmented to white at the first transfer. After about six weekly transfers, the remaining pigmented colonies were stable, i.e., they did not revert from pigmented to white. When tested for virulence, the pigmented colonies were virulent. Isolating one of these EP-11 derived strains from a virulent canker and plating the conidia resulted in 100 percent pigmented colonies. However, when conidia from one of these colonies were irradiated with UV light sufficient to kill 99 percent of the conidia, 1-10 percent of the surviving conidia yielded white colonies. This ratio of white to pigmented colonies is much higher than one would expect from genetic mutation. When these white colonies were tested for virulence they formed typical hypovirulent cankers. Hypovirulent colonies are not always obtainable from virulent strains derived from EP-11. In those cases, the hypovirulent factor has evidently been lost.

INSTABILITY OF EP-49

Evidently, in some ostensibly normal strains derived from unstable hypovirulent ones the hypovirulent factor is still present but somehow suppressed. The strains reported by Elliston (1977; 1979 and Day *et al.* (1977) to be "hypovirulent" and yet virulent in canker production may be similar to the suppressed hypovirulent strains that we studied.

One of the dsRNA containing virulent strains obtained from Elliston (EP-49) yielded all pigmented colonies when conidia were plated onto PDA. If conidia were irradiated with UV light, however, from 1-20 percent of the surviving colonies were white. A selection of white and pigmented colonies was made and sent to the Connecticut Agricultural Experiment Station, New Haven, where they were tested for virulence and presence of dsRNA. The results clearly showed that this pigmented virulent strain containing dsRNA was unstable and yielded white strains containing dsRNA. The white strains produced typical hypovirulent type cankers in chestnut trees. (The cankers produced by white strains were 116 ± 59 mm^2 while the cankers of pigmented strains were $2,700 \pm 379$ mm^2 in area.) These white strains, much like EP-11, were unstable and when irradiated with UV light the ratio of white to pigmented colonies was reduced.

SUPPRESSED HYPOVIRULENCE

The data presented here suggest that hypovirulence can exist in a suppressed state. This type of strain is virulent when tested for pathogenicity, and thus should not be referred to as hypovirulent. However, either UV irradiation or, in some cases, inherent instability removes the suppression and allows expression of hypovirulence. Since such strains are neither typically normal nor typically hypovirulent, we propose that they be termed suppressed hypovirulents (5).

One of the strains, EP-49, which evidently has a suppressed hypovirulent state was isolated from nature in Italy. The existence of such strains in nature should be of concern to us in our attempts to introduce hypovirulence into American virulent strains of *E. parasitica* in the field. Considering the phenomenon of cross-protection, we should be wary of a strain that possesses the hypovirulent factor and yet is every bit as virulent as a normal strain. Possibly the reduced sporulation of this strain observed by Elliston (1977; 1979) renders it less competitive, but it was isolated from nature, therefore it has survived.

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