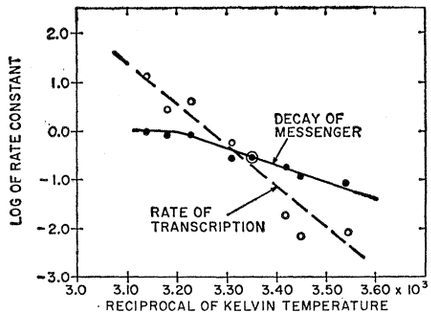

Fig. 4. The logarithm of the decay constant for messenger RNA and the rate constant for transcription of message are plotted against the reciprocal of the absolute temperature. The activation energy for decay is 11,000 cal/mole, while that for transcription is 22,000 cal/mole.

constant, so that $(d^2E/dt^2) = 0$, we have $ab = k_1(dE/dt))m$. Thus we can find the quantity ab dependent on the measurement of E being in arbitrary units. If we follow the suggestion that the basal amount of enzyme in a fully repressed culture corresponds to one molecule of messenger RNA per cell, we can make the arbitrary units indicative of the numbers of transcribed molecules per unit of time. This has been done by measuring the number of cells in each experiment, and also by measuring the amount of enzyme from a known number of fully repressed cells with exactly the same assay procedure. We designate these units as "basal units" and hope that one basal unit is equivalent to one molecule of messenger RNA.

In Table 1 we give numbers found in this way for different temperatures. In Fig. 4 we show the Arrhenius graphs [in which the relation $\ln(\text{rate}) = -(\Delta H^*/RT)$ is used, where ΔH^* is the energy of activation, R the gas constant and T the absolute temperature] for the decay of messenger and also for the rate of transcription. For the decay of messenger, ΔH^* is found to be 11,000 calories per mole, while that of the rate of transcription is 22,000 calories per mole. Both are in a reasonable range for enzyme action; although the transcription value is rather high.

The variation of the decay constant with temperature is further evidence that the decay is some kind of enzymatic degradation rather than physical inactivation analogous to thermal inactivation. Although the points do lie sufficiently on the line, the individual variations in decay constant can be as high as a factor of two. This suggests that the metabolic state, which is altered by, for example, good aeration, may also affect the decay, so that temperature is only one of several variables of importance.

The fact that very reasonable figures for the decay of messenger over a wide range of temperature, there being good agreement with good measurements made quite differently, are obtained by assuming that radiation acts to stop transcription forces consideration of this as a hypothesis. Such a hypothesis is supported by experiments of Pollard and Achey (*13*) in which it was found that in the presence of oxygen the DNA of *E. coli* is degraded to the extent of 50 percent, but no more, and also that there is a reduction in the synthesis of DNA after irradiation. If it were supposed, as a hypothesis, that the 50 percent degradation took place in one strand of DNA, which is the strand which is transcribed, then a natural explanation of a great reduction of transcription is at once available. In addition, if it is supposed that the new synthesis of DNA is temporarily halted by radiation, then no new DNA is available to be transcribed, and so there is a stoppage. It has been suggested, for quite different reasons, by Champe and Benzer (*14*), Bautz (*15*), and McCarthy and Bolton (*16*), that only one of the two strands of DNA is transcribed. The DNA is presumably in a different physical state while the process of transcription is going on. It would appear that the two effects of radiation mentioned above are sufficient to stop the process of transcription. Since this type of inhibition of transcription, regardless of the hypotheses advanced to explain it, is not specific at all, it should be possible to exploit it to make a general study of a wide variety of messenger RNA half-lives and also, possibly, of rates of transcription.

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Ceratocystis Infection in Sweet Potato: Its Effect on Proteins, Isozymes, and Acquired Immunity

Abstract. *Changes take place in the protein and isozyme patterns of tissue adjacent to cut surfaces of sweet potato roots infected by the fungus* Ceratocystis fimbriata. *Chromatography and gel electrophoresis of extracts from sections cut at known distances from a plane of infection showed that inoculation with a pathogenic or nonpathogenic isolate produced similar changes in several proteins and enzymes. Inoculation of a susceptible variety of sweet potato with the nonpathogenic isolate induced in a thin layer of tissue around the site of inoculation an acquired immunity to subsequent inoculation with the pathogen.*

Changes in protein metabolism have been associated with black rot infection of sweet potatoes; the protein content (*1*), polyphenol oxidase activity (*2*), and peroxidase activity (*3*) increase in the tissue adjacent to the site of infection. This suggests that more detailed analysis of the tissue adjacent to sites of infection would contribute to the understanding of the physiology of the diseased plant. We have used chromatography and gel electrophoresis in studies of resistant and susceptible sweet potatoes interacting with pathogenic and nonpathogenic isolates of the fungus *Ceratocystis fimbriata* (E. H.) Ell.

Resistant (Sunnyside) and suscepti-

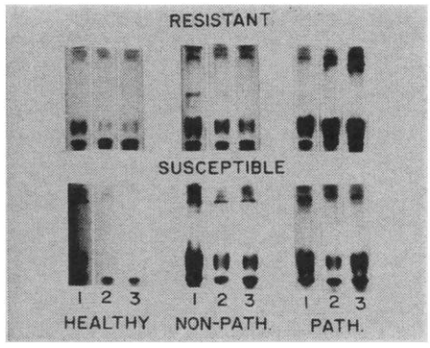

Fig. 1. Peroxidase activity of resistant (Sunnyside) and susceptible (Jersey Orange) sweet potato roots. The sets of three represent separation by gel electrophoresis of the soluble fraction of healthy tissue, tissue inoculated on the surface with a nonpathogenic strain of *Ceratocystis fimbriata*, and tissue inoculated on the surface with a pathogenic strain of *C. fimbriata*. The numbers *1, 2,* and *3* represent the distance in millimeters below the cut surface of the healthy sweet potato or the boundary between the infection plane and underlying tissue.

ble (Jersey Orange) varieties (*4*) of sweet potato, *Ipomea batatas* L. (Lam.), were used (*5*). Individual roots of both were cut crosswise into 2-cm sections. The cut surfaces were inoculated with mycelial fragments of the pathogenic or nonpathogenic isolate of *C. fimbriata*. For controls we used roots of both strains which were not inoculated with *C. fimbriata*, but were treated with sterilized culture medium otherwise similarly treated. The sections were incubated in sterilized glass chambers (90 mm by 50 mm) at 25°C and about 90 percent relative humidity. In 3 to 4 days, aerial mycelium of the pathogenic isolate completely covered the cut surfaces of both resistant and susceptible roots. Subsequent growth on tissue of resistant roots was, however, confined to the surface layer; on susceptible tissue mycelium penetrated and rotted the underlying tissue. With the nonpathogenic isolate there were only traces of visible mycelium on either resistant or susceptible tissue, with indications that the fungus had made some initial growth and then stopped.

Four days after inoculation, when fungal penetration was not visible below 1 mm, the sections were cut normal to the surface with a cork borer (18-mm diameter). The individual tissue cylinders were then cut with a freezing microtome into disks (1 mm thick) parallel with the infected surface. The upper infected sections containing the fungus were discarded; disks were selected outward from the boundary between the infected plane and the underlying tissue. The tissue disks were immediately placed in 0.01*M* tris-hydrochloride buffer, *p*H 7.4, containing 1 percent ascorbic acid and 12.5 percent sucrose, and cooled with dry ice. From each type of sweet potato we obtained 20 disks from each variously treated layer—that is, from tissue inoculated with the pathogen or with the nonpathogenic isolate, and from control tissue treated only with sterilized culture medium. The disks of each lot were forced through the fine orifice of a sodium press to disrupt the cells. Nitrogen was bubbled through the solutions and cylinders; the complete operation was conducted in the cold room at 4°C. The broken tissue and buffer was then centrifuged at 20,000*g* for 20 minutes; the supernatants were frozen in small vials until used. Nitrogen content of the samples was determined by a modified Nessler reaction (*6*).

The samples (adjusted to similar concentrations on the basis of nitrogen content) were separated by gel electrophoresis (*7*) at 4°C, and then incubated with specific substrates and cofactors to detect peroxidases, oxidases (polyphenol oxidase), acid phosphatases, dehydrogenases, and esterases (*8*).

Proteins in extracts from the third layer of tissue in each treatment group were separated on a diethylaminoethyl cellulose (200 to 325 mesh, 0.85 meq/g) column (0.5 cm² by 58 cm) by a gradient developed by Woodbury (*9*). The samples were eluted first by 60 ml of 0.01*M* tris-hydrochloride buffer, *p*H 7.2, and then by a salt gradient (19 ml of 5*M* NaCl and 300 ml of 0.01*M* tris-hydrochloride, *p*H 7.2) at 20 ml per hour. The eluent was monitored at 220 mμ and the change in gradient determined by conductivity. Chromatograms of extracts from the resistant host after inoculation with the nonpathogenic or pathogenic isolate were very similar. Compared with extracts from control roots, several changes were evident in extracts from tissue which had been inoculated with either isolate. Some of the peaks on the chromatograms increased, while others decreased. It was concluded that the nonpathogenic isolate induced responses in the susceptible host that were similar to the responses induced by the pathogenic isolate.

The enzyme activity of the layers was assayed by gel electrophoresis and enzyme assays. The pattern of peroxidase activity as shown in Fig. 1 indicates that in the healthy tissue there was stimulation of peroxidase at the sliced surface (first layer), with but little peroxidase activity in the second and third layers. In contrast, inoculation with the nonpathogenic or pathogenic isolate induced high peroxidase activity in both underlying layers. The resistant host appeared to be the more responsive to the pathogenic isolate. The Nadi reaction was used to detect polyphenol oxidase, peroxidase, amine oxidase, and cytochrome oxidase. Studies with inhibitors and substrates indicated that most of the bands appearing with the Nadi reaction in extracts of sweet potatoes were due to polyphenol oxidase activity; increase in this activity occurred in the underlying tissue of the resistant and susceptible hosts after inoculation with the nonpathogenic or pathogenic isolate. The data were interpreted as evidence that both the isolates stimulate increased enzyme

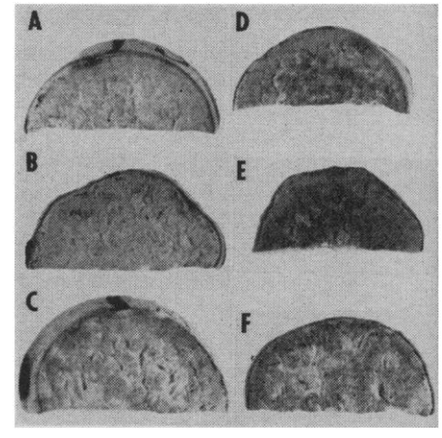

Fig. 2. Acquired immunity or resistance of sections (2 cm thick) of susceptible (Jersey Orange) sweet potato to a pathogenic isolate, induced by prior inoculation with a nonpathogenic isolate of *Ceratocystis fimbriata*. *A*, No prior inoculation (control). *B*, Treated with sterile culture medium. *C*, Inoculated with a nonpathogenic isolate of *C. fimbriata*. *D*, Same as *A*, but inoculated with only a pathogenic isolate of *C. fimbriata*. *E*, Same as *B*, but challenged 2 days later with a pathogenic isolate. *F*, Same as *C*, but challenged 2 days later with a pathogenic isolate. Heavy growth of the pathogenic fungus occurred on the sections that were treated with the sterile culture medium or were merely incubated, before being inoculated with the pathogen, whereas the section that had been inoculated with the nonpathogenic isolate had little growth following challenge with the pathogenic isolate.

activity several millimeters below the infection plane.

In contrast with these observations, acid phosphatases decreased following inoculation with either isolate; activity decreased proportionately from the surface, but the decrease was more pronounced with the pathogenic isolate. Another acid phosphatase of fungal origin was detected in tissue inoculated with the pathogenic isolate; activity was highest in the first layer. It was concluded that the acid phosphatase of the host was inactivated in the first layer, whereas the fungal (pathogenic) acid phosphatase retained activity.

Malic dehydrogenase activity decreased proportionately from the surface following inoculation with nonpathogenic or pathogenic isolates. Similar changes in esterase activity were observed in the susceptible host; in the resistant host, activity was very low and difficult to detect.

The increases and decreases in enzyme activity inferred that the fungus induced changes in the host's protein synthesis or enzyme activities several millimeters away from the site of infection. The similarity in the changes induced by the nonpathogenic isolate to those by the pathogen suggested that inoculation with a nonpathogenic isolate might cause changes in a susceptible host that would induce immunity or resistance.

To examine this possibility, sweet potatoes (susceptible) were cut into sections 2 cm thick and divided into three groups. One group was incubated without treatment, a second was treated with sterile culture medium, and the remainder were inoculated with mycelial fragments of the nonpathogenic isolate of *C. fimbriata*. The following day, sections from the three groups were inoculated with mycelial fragments from the pathogenic isolate. Other sections from each group were also inoculated with the pathogen on additional days (up to 6 days). The results (Fig. 2) indicated that the earlier inoculation with the nonpathogenic isolate did indeed produce an acquired immunity or resistance toward the pathogen; immunity or resistance was limited to a few cell layers from the plane of inoculation with the nonpathogenic isolate. The immunity could be induced on only one side of a section and to some extent on one half of the same side. This suggested that the acquired immunity was a local response related to a stimulation by the nonpathogenic isolate of the host's defense mechanism in those few cell layers immediately adjacent to the surface. When this resistant layer was removed by scraping just before the second inoculation, the tissue became susceptible. Likewise, cutting through this resistant layer allowed the fungus to reach the underlying susceptible tissue and to grow. The sections that were cut only, treated with sterile culture medium, or inoculated with the nonpathogenic isolate, all developed callus on the surface. All subsequent inoculation with the pathogenic isolate on surfaces which had been cut only, or had been treated with sterile culture medium, resulted in infection, whereas surfaces which had been previously inoculated with the nonpathogenic isolate remained resistant or immune. This suggests that callus formation alone was not the cause of resistance. Filtrates or homogenates of cultures of either isolate did not induce the immunity or resistance when applied to the cut surface.

The concept of acquired immunity has been associated with phytoalexin formation (*10*), but acquired immunity without association with a phytoalexin has been reported (*11*). In sweet potato, Uritani has associated resistance with ipomeamarone formation (*2*) which has been classed as a phytoalexin (*12*). Ipomeamarone formation does not seem to account for the induced immunity reported here; ipomeamarone could not be detected with Ehrlich reagent in the tissue inoculated with the nonpathogenic isolate whereas it was easily detected during this same period in the tissue inoculated with the pathogen. Mueller (*10*) treated white potatoes with an avirulent strain of *Phytophthora* and obtained resistance to a virulent strain; resistance was associated with a necrotic reaction. In contrast, the acquired immunity to *C. fimbriata* in sweet potato was not associated with necrotic reaction.

Evidence from this investigation would favor an explanation of the mechanism of resistance in terms of altered protein synthesis or enzyme activity. The stimulus for this change has been shown to be supplied by a nonpathogenic fungus. The rate of response to such a stimulus may be a factor which determines whether a host is resistant or susceptible. Alterations in enzymes and isozymes have been observed in other plant diseases (*13, 14*). Whether changes in isozymes in plants play a role in plant diseases is yet to be established, but the changes observed in this investigation would appear to favor this concept.

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Crustacea: A Primitive Mediterranean Group also Occurs in North America

Abstract. *A new species of the genus* Monodella *(class Crustacea, order Thermosbaenacea) has been found in a cave pool in Texas. Previously the order was believed to be restricted to the Mediterranean area. The new evidence indicates that the order is older than was believed, or that invasion of fresh or brackish water has occurred more than once within the order.*

Until the recent discovery of a new species in Texas, the primitive crustacean order Thermosbaenacea was throught to be restricted to the area of the Mediterranean Sea. These small crustaceans (1 to 3 mm long) lack