agreement with the newly organized Mayo Forestry and Horticultural Institute whereby several tracts of land will be made available to the university for research and demonstration in forestry and horticulture. The Mayo Properties Association has appropriated $25,000 for tools, labor and planting materials. The forestry work will occupy about 140 acres of land in or near Rochester, Minn., mostly on property of the Institute of Experimental Medicine, and will include studies on erosion control in cooperation with the Soil Conservation Service of the U. S. Department of Agriculture. The university division of horticulture plans to use about 40 acres of this tract, originally developed as an orchard under private ownership prior to its donation in 1943 to the Mayo Properties Association. Variety tests and demonstrations with apples are to be continued and enlarged, and several acres will be used for small fruit plantings. W. H. Alderman, chief of the division of horticulture, has been given charge of the fruits projects, and Dean Henry Schmitz, of the College of Agriculture, Forestry and Home Economics, of the forestry areas.

SPECIAL ARTICLES

LYCOPERSICIN, A FUNGISTATIC AGENT FROM THE TOMATO PLANT

FUSARIUM wilt, caused by *Fusarium oxysporum* f. *lycopersici* (Snyder and Hansen),[1] is one of the most prevalent and damaging diseases of tomatoes in many regions of the United States. The mechanism of the wilting caused by this vascular parasite is obscure, but wilting of tomato plants infected with *F. oxysporum* f. *lycopersici* (hereafter designated Fol.) can be correlated with the presence in the tracheal fluid of the host of a toxin which is presumably elaborated by the fungus.[2] Since tomato varieties vary in their susceptibility to Fusarium wilt[3] it might be postulated variously that (1) certain varieties are wilt-resistant because they are able to produce a substance or substances which either neutralize the toxin directly or inhibit the growth of the fungus; (2) certain varieties are susceptible because they produce a substance or substances that promote or make possible growth of the parasite, or (3) differences in susceptibility or resistance are due to differences in amounts of such substances common to both categories of plants. Fisher[4] and more recently Gottlieb[5] have obtained evidence to indicate that the expressed juice from tomato plants retards growth of Fol. in culture in proportion to the wilt-resistance of the tomato varieties tested.

In this laboratory we have obtained from the expressed juice of Pan America tomato plants, a variety which exhibits a high degree of wilt resistance,[6] a preparation which, though still impure, possesses marked fungistatic activity toward Fol. This antibiotic agent, which will be designated "lycopersicin," occurs throughout the mature plant. In the crude preparations available, lycopersicin is completely stable, as indicated by assay, for at least 1 hour at 100° C and withstands autoclaving for at least 15 minutes at 15 pounds pressure. It is dialyzable (Visking, Cellophane membrane), adsorbed from aqueous solution at pH 5.5 on charcoal (unactivated Norit A), soluble in water and methanol, partially soluble in ethanol, and insoluble in chloroform, acetone, ethyl acetate, ether, petroleum ether and benzene.

A rapid and accurate method for the assay of the fungistatic activity of lycopersicin, patterned after the cylinder-plate method now used for the assay of penicillin and related antibiotic agents,[7] has been developed. Sterile, 90 mm Petri dishes, containing 20 ml of solidified Czapek-dextrose agar,[8] are warmed to approximately 45° C. and flooded with 3.5 ml of a suspension of spores of Fol.[9] in the same medium. To prepare the inoculum 5 ml of sterile distilled water are added to a 5-day-old potato-dextrose slant of the organism and the surface is gently stroked with a sterile loop to yield a heavy aqueous suspension of spores. The suspension is filtered through a thin, sterile cotton plug (to remove bits of mycelium which, if allowed to remain, give rise to growth irregularities on the assay plate) into 40 ml of Czapek-dextrose agar maintained at 45° C. After thorough mixing, the inoculum is flooded evenly over the agar surface in the Petri dish and is allowed to solidify. Porcelain

[1] Synonymous with *Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and R.

[2] D. Gottlieb, *Phytopathology*, 34: 41, 1944.

[3] W. S. Porte and F. L. Wellman, *U. S. Dept. Agr. Circ.* No. 584: 1941.

[4] P. L. Fisher, *Maryland Agr. Expt. Sta. Bul.* 374: 1935.

[5] D. Gottlieb, *Phytopathology*, 33: 1111, 1943.

[6] W. S. Porte and H. B. Walker, *U. S. Dept. Agr. Circ.* No. 611: 1941.

[7] W. H. Schmidt and A. J. Moyer, *Jour. Bact.*, 47: 1, 1944.

[8] $NaNO_3$, 3.0 g; $K_2HPO_4 \cdot 3H_2O$, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; dextrose, 30 g; agar, 20 g; water, 1,000 ml.

[9] The culture used throughout this work was a transfer from the R-5-6 strain of Wellman (*Phytopathology* 32: 271, 1942) that was originally selected for its high pathogenicity. At the start of the present investigation a large stock of lyophil tubes of spores of this organism was prepared (see method of Raper and Alexander to be published in *Mycologia*, July-August, 1945) from vigorously growing potato-dextrose agar slants. To obtain the spores necessary for preparing inoculum for the assay plates potato-dextrose slants are inoculated weekly from a newly broken lyophil tube. In this manner the source of inoculum for the assay plates is maintained constant both as to age and virulence and the danger of introducing contaminants is minimized.

cylinders of the type used for penicillin assay (8 mm outside diameter, 10 mm high)[10] are dropped on the inoculated surface and the covered plate is incubated for 24 hours at 28° C. in a constant temperature incubator equipped for continuous air circulation. The solutions to be assayed are pipetted into the cylinders and the plates are incubated for an additional 16 hours under the same conditions, after which the inhibition zones are measured.

Growth of the fungus on plates prepared in this manner is uniformly raised, white and cottony, and develops evenly and spontaneously over the entire agar surface. Inhibition zones surrounding the cylinders are sharply defined and can be measured accurately. Control cylinders, filled with water, show no evidence of inhibition. Plates to which the test solution is added before 20 hours or after 30 hours of incubation show less distinct inhibition zones than those to which test solutions are added after the optimum period of incubation, namely, 23 to 25 hours after inoculation. Inhibition zones must be measured within 15 to 17 hours after the test solutions have been applied. After periods of less than 15 hours the inhibition zones are not well defined due to the thinness of the fungus mat; after more than 17 hours, overgrowth of the edges of the inhibited zones may occur.

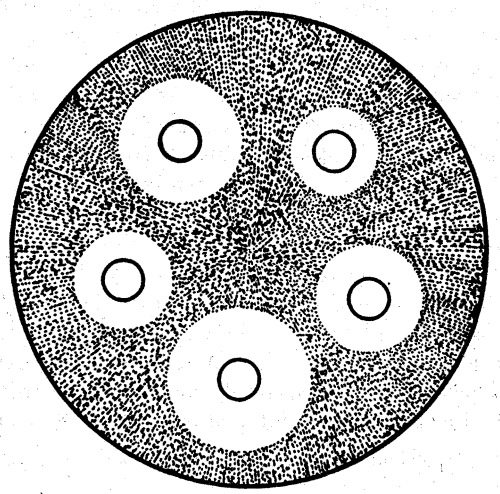

FIG. 1. Sketch of typical lycopersicin assay plate showing inhibition zones produced by several dilutions of the standard solution. Reading clockwise from the bottom the cylinders contain 10, 1, 5, 0.5 and 2 lycopersicin units per ml, respectively.

A typical assay plate illustrating the gradation in response produced by dilutions of an arbitrarily established standard solution[11] is shown in Fig. 1. The standard solution and 19 dilutions of the standard solution were assayed repeatedly and the average diameters of the inhibition zones produced by each concentration of the standard were used in constructing the standard curve shown in Fig. 2. The diameter

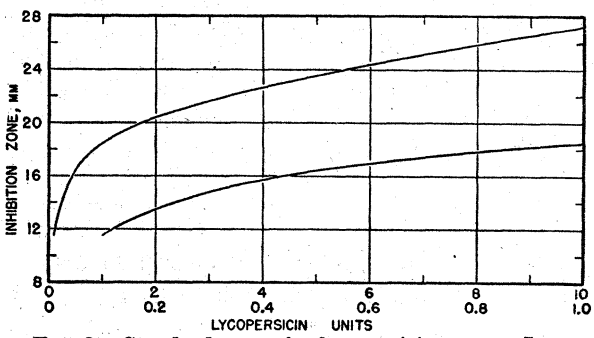

FIG. 2. Standard curve for lycopersicin assay. Lower curve represents an expanded plot of the left hand portion of the upper curve.

of the inhibition zone produced by a given solution was found to be reproducible within narrow limits. For example, the diameters obtained in replicate tests on different plates for one of the solutions used were 23.2, 23.9, 23.7, 24.0, average 23.7 mm. In constructing the curve shown in Fig. 2 one of the dilutions of the standard solution was arbitrarily considered to contain one lycopersicin unit per ml and points for other dilutions of the standard solution were plotted accordingly. Under the conditions described a solution containing one unit of lycopersicin per ml will produce an inhibition zone of approximately 18.5 mm. Comparable assays can be obtained using this arbitrary standard until the isolation of lycopersicin makes it possible to evaluate the unit in terms of the weight of pure compound required to effect equivalent inhibition.

It will be realized that at least a part of the standard curve represented in Fig. 2 must be redetermined for each series of assays to compensate for variations in the standard response. The curve in Fig. 2 is only representative of a family of curves having the same shape but in which the individual curves may lie slightly above or below the one shown. Accuracy of assays by this technique appear to be of the same order of magnitude found for the assay of penicillin[7] and the method is subject to similar limitations.

By use of the standard and standard curve described it has been possible to assay the various parts

[10] Fisher Scientific Company, "Penicylinders."

[11] The standard solution was prepared as follows. Thoroughly washed, mature Pan American tomato plants (115 days old), including the roots but not the fruit, were dried, finely ground and extracted by refluxing with methanol. The methanol extract was concentrated to dryness, the residue was extracted with water and the aqueous extract was lyophilized to dryness. A dilute aqueous solution of the dry material was distributed in 1.5 ml portions among several Pyrex ampoules and the sealed ampoules were autoclaved for 15 minutes at 15 pounds pressure. This standard solution appears to be stable indefinitely when stored in the refrigerator.

of different tomato varieties for lycopersicin activity at all stages of growth from the seed to maturity. For this purpose the mechanically expressed, sterilized juice or sterile aqueous extracts of the plant tissue are placed directly in the assay cylinder. Sufficient data have not been obtained as yet to justify definite conclusions regarding the relative amounts of lycopersicin present in the materials which have been assayed; but it may be concluded that: (a) of the tomato varieties tested, including Bonny Best (highly susceptible to Fusarium wilt), Rutgers and Marglobe (resistant), Pan America and Red Currant (highly resistant), all contain the inhibitor; (b) lycopersicin activity, while absent in the seed, appears in seedlings germinated in the dark and in the plant within 8 days after planting; (c) the concentration of lycopersicin varies somewhat with the age of the plant and considerably with the plant part assayed. Results of these investigations, as well as consideration of the relationship between lycopersicin and the Fusarium wilt of tomatoes and consideration of the specificity of lycopersicin will be reported elsewhere.

The authors wish to express their appreciation to Dr. O. E. May, chief of the Bureau of Agricultural and Industrial Chemistry, for suggesting these investigations. Thanks are due Dr. K. B. Raper, Mr. W. H. Schmidt and Miss Dorothy F. Alexander for their cooperation and advice; and to Mr. O. W. Eady for technical assistance.

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ASPERGILLUS USTUS

THERE are now in the literature several reports of antibiotics active *in vitro* against *Mycobacterium tuberculosis*. They have been derived from culture filtrates of a variety of molds including *Aspergillus fumigatus*,[1,2,3] *Actinomyces griseus*[4,5] and one of the Penicillium group.[6] The present report will describe yet another.

Early in 1944, while systematically examining the antibiotic-producing properties of a number of fungi appearing as contaminants on routine culture plates, we came upon one which made its culture medium highly active against *M. tuberculosis*. It has subsequently been identified[7] as a strain of *Aspergillus ustus*, (Bain) Thom and Church, and examined more completely for its several properties.

The mold grows well on Czapek-Dox medium with 4 per cent. glucose and 0.1 per cent. Bacto-Yeast extract added. After 36 hours' culture there appears on the surface of the medium a thin veil-like growth which on the following day or two assumes a pale blue-green color. As the spores begin to develop the growth becomes more intensely green, and ultimately forms a brown wrinkled membrane. Frequently one may observe numerous clear yellow droplets on the surface of the culture. The temperature for the optimal production of the antibiotic substance appears to be 28° C., but the fungus will grow over a wide range, including 37° C. With the progressive growth of the culture there is a gradual increase in pH of the medium from an initial 5.8 to a final value between 8.0 and 8.4.

The substance that inhibits the growth of *M. tuberculosis* can first be demonstrated on the sixth day of culture and continues to increase to a maximum concentration at 14 to 16 days. It can be extracted from the medium by the use of various solvents, such as ether, chloroform, acetone, or by adsorption onto Norit, followed by elution with ether. Extraction with ether at pH 8.0 to 8.4 yields a light yellow amorphous residue which is insoluble in water but soluble in either 1 per cent. sodium carbonate solution or 95 per cent. alcohol. The potency of this ether-extracted residue on the tubercle bacillus was determined by preparing serial dilutions of the dry crude residue in Long's synthetic medium and then planting on this medium a thin surface growth, approximately 7 mm in diameter, of *M. tuberculosis*, Strain H37. When examined after an incubation period of thirty days at 37° C., the tests usually showed complete inhibition of growth in dilutions varying from 1:200,000 to 1:400,000, but the activity of this ether extracted residue varied with each batch of substance tested. The controls, prepared in the same manner but without the addition of the residue, showed a heavy growth covering the entire surface of the medium at the end of thirty days. It is interesting to note that similar experiments conducted with *Mycobacterium ranae* showed that the growth of this organism was inhibited to the same extent as that of

[12] Division of Biologically Active Compounds, Bureau of Agricultural and Industrial Chemistry.

[13] Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering.

[1] A. Vaudremer, *C. R. Soc. Biol.*, 73: 51, 1912; 74: 278 and 752, 1913.

[2] M. A. Soltys, *Nature*, 154: 550, 1944.

[3] Igor N. Asheshov and Frieda Strelitz, SCIENCE, 101: 120, 1945.

[4] S. A. Waksman, *Proc. S.N. Mayo Clinic*, 19: 537, 1944.

[5] S. A. Waksman and A. Scholz, *Ibid.*, 57: 244, 1944.

[6] D. K. Miller and A. C. Rekate, SCIENCE, 100: 172, 1944.

[7] A culture of the fungus was sent to Dr. Charles Thom, and we are grateful to him for the above classification.