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Crown Gall-Like Tumors Induced with Scharlach Red on the Plant, Kalanchoe.

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The reaction of plants to carcinogenic agents, growth substances, vitamines, and heteroauxins has been intensively studied by a number of workers during the past few years. Primarily, their purpose has been to determine the physiological responses made by various garden and house plants to these chemical substances. Other botanists have been interested in determining whether these chemical agents were capable of producing overgrowths analogous to crown gall, induced by *Pseudomonas tumefaciens,* which has been considered cancer[1] of plants, and which they contended were similar to cancer in animals and man.

In earlier reports,[2, 3, 4] the writer has shown that a large number of chemical substances produced small proliferations on susceptible plants but which lacked the essential characteristics of crown galls. In a category of neoplastic diseases the position of these overgrowths was of the lowest order, in that their cell multiplication was limited, differentiation of the induced meristematic activity was rapid and reached its final stage in development before it had developed into a sizable mass. Crown galls on similar plants, grown under similar conditions, and produced by inoculation with a virulent strain of the bacteria, were of a higher grade than the chemical plant "tumors", while cancer in animals and man was the highest order because of its limitless growth and malignancy. Studies on plants made with the heteroauxin, indole acetic acid, have shown that while these plants responded by producing an abundance of roots over the treated areas, swellings also occurred; and where the applications were made on decapitated bean seedlings, the cut surface produced clusters of intumescences which have been described as "tumors".[5, 6, 7]

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1 Smith, E. F., Brown, N. A., and Townsend, C. O., *U. S. Dept. Agr. Bull.*, 1911, **213**, 1.

2 Levine, M., *Bull. Torrey Bot. Club*, 1934, **61**, 103.

3 Levine, M., *Bull. Torrey Bot. Club*, 1936, **63**, 177.

4 Levine, M., and Chargaff, E., *Am. J. Bot.*, 1937, **24**, 461.

5 Brown, N. A., and Gardner, F. E., *Phytopathology*, 1936, **26**, 708.

6 Kraus, E. J., Brown, N. A., and Hamner, K. C., *Bot. Gaz.*, 1936, **98**, 370.

7 Link, G. K. K., Wilcox, H. W., and Link, A. DeS., *Bot. Gaz.*, 1937, **98**, 816.

A study[8] of the effects of scharlach red on the *Lycopersicum esculentum,* of the cherry stone variety, showed, on microscopic examination, abundant hyperplastic tissue and some hypertrophy. These suggested the possibility of continued growth under more suitable conditions. In view of the continued favorable results obtained with scharlach red in producing cancer in guinea pigs, rats, and mice reported by Yoshida,[8] Shear,[9] and others, it appeared advisable again to include this dye in further studies on plants. At the same time the 3 principal carcinogenic hydrocarbons, namely, benzpyrene, methylcholanthrene, and 1,2,5,6-dibenzanthracene, were again tested.

During the last 2 years a large number of species of garden and house plants were grown in the open, and under more sheltered conditions. *Kalanchoe Daigremontiana* (Hamet et Perrier) was found to produce crown gall-like overgrowths, with leafy shoots and roots, after a single application of scharlach red suspended in ether. The nature of these masses is so much like those induced by *P. tumefaciens* that it seems desirable to call attention to them now.

The amount of chemicals for each plant was not determined. A paste consisting of 1% to 2% of the chemical thoroughly mixed with lanolin was applied by means of a glass rod. Each plant was treated with scharlach red (0.2 g suspended in 20 cc of purified ether) by a single painting with a camel's hair brush. The controls were on the same or other plants of the same species treated in the same manner but with lanolin (hydrous) only. The procedure consisted of decapitating the growing or apical portion of the plant down to a point somewhat below the middle of the second or third internode. The cut portion of the stem was further injured by pricking it with a sterile, steel needle. More than 50 plants were used in these tests.

Observations. Decapitated stems of Kalanchoe treated with 1% scharlach red in ether produce an abundance of roots after 10 to 12 days. Most of these adventitious roots appear to arise from the node, while the remnant of the severed internode shows slight swelling about the treated surface. The roots increase in number for several days, forming long matted structures. The roots are white and measure more than an inch in length and .25 mm to .5 mm at the base. As the roots grow old they become twisted, reddish brown in color, at the same time, new, white roots make their appearance. At about 25 days after the treatment, the short, internode stub showed a small, smooth, globular mass about the size of a pea above the cluster of roots; this grew rapidly. In the meantime, one of the

[8] Yoshida, T., *Gann,* 1934, **28**, 454.

[9] Shear, M. J., *Am. J. Cancer,* 1937, **29**, 269.

axillary buds alongside the mass developed into an apparently normal shoot.

At approximately 50 days after treatment (Fig. 2), the globular mass developed a number of smooth, confluent bodies with leaf-like projections from the upper surface and roots from its lower part. These galls are faintly green to a yellowish white in color. Streaks of red pigmented tissue are seen in the lower glaucous, under surface of the largest mass. One of these tumors, more than 6 months after treatment is shown on a Kalanchoe in Fig. 1. Here a typical crown gall-like structure is seen with leafy shoots and with an abundance of old and young roots. The lower part of the overgrowth is studded with many protruding, thick, conical structures which appear to be modified root-like organs. These structures are faintly pink in color.

On removing a part of one of the galls for microscopical studies, the macroscopical appearance of the cut surface was indistinguishable from gross sections of crown gall tissue produced in this and other species of plants by the bacteria. The tissue generally is white with deposits of chlorophyll near the abortive, leafy structures; the latter are intensely green. It appears, from the macroscopical structure of these chemically formed galls, that they are identical with those produced by *P. tumefaciens.* The ultimate fate of these scharlach red tumors or their effects on the host are unknown.

Kalanchoes treated with the heteroauxin, indole acetic acid (3% mixture) show essentially different responses as in Fig. 4. The decapitated internode, when covered with a paste of this agent, becomes covered with long, white roots which persist for over a month. In other cases when the internode is covered with the heteroauxin, nodular masses occur with short, stubby roots. These masses necrotize, and at the same time the upper axillary buds develop into normal active shoots. The roots in the former instance wither; they mat themselves about the stem, which frequently cracks, and form only small nodules or intumescences. The tip of the excised internode produces a normal shoot. No tumors that are comparable with crown gall result.

The application of a 2% 1,2,5,6-dibenzanthracene in a lanolin paste to the cut end and surface of the decapitated internode portion of the Kalanchoe, invariably produces an abundance of persistent roots over the treated area. These are thick in structure and white in color. They persist for many months, branch, and become brown, yet young freshly formed white roots appear among them (Fig. 3). Thickened scars have been observed over the treated areas on similar

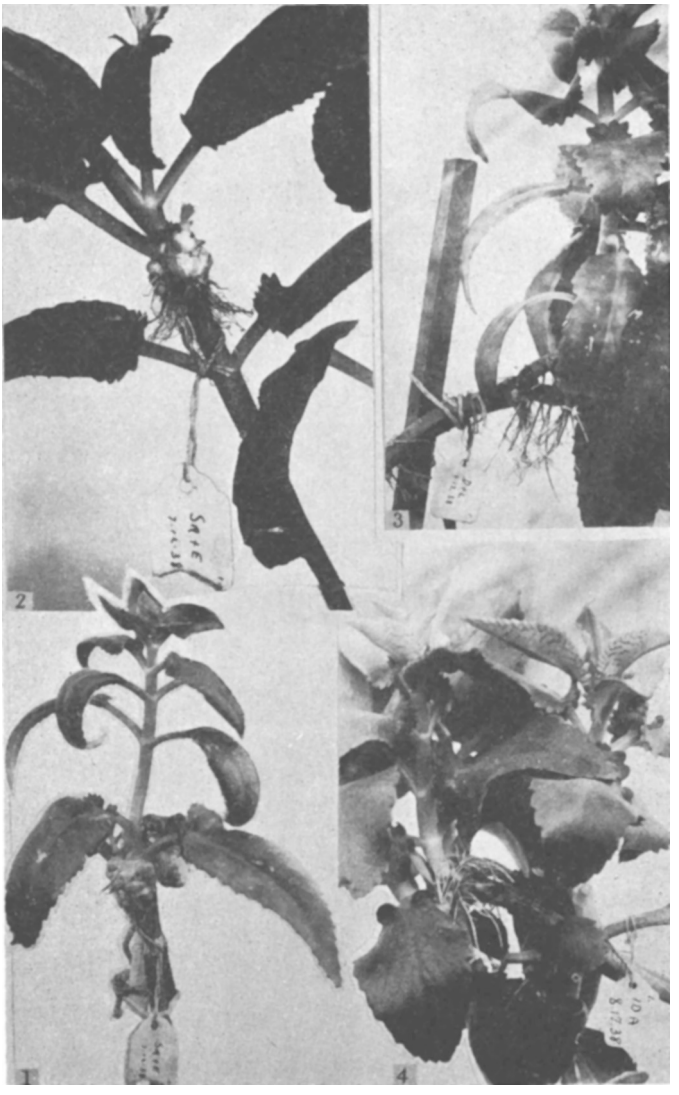

FIG. 1.
Crown gall-like tumor on Kalanchoe; stem treated with 1% scharlach red on 7-12-1938; photographed 1-20-1939. × ½.

FIG. 2.
Early stage of the same tumor; photographed 8-29-1938. × 1½.

FIG. 3.
Kalanchoe treated with 2% 1,2,5,6-dibenzanthracene in lanolin 7-12-1938; photographed 1-20-1939. This plant shows no tumor, instead roots. × ½.

FIG. 4.
Kalanchoe treated with 3% indole acetic acid in lanolin 8-17-1938; photographed 1-20-1939, shows only roots. × ½.

plants. Normal shoots develop from the edges of the cut surfaces of the internodes. No tumors have been observed on the Kalanchoes so treated.

A 1% paste of either methylcholanthrene or benzpyrene applied to Kalanchoes frequently caused necrosis of the internode remnant, down to the node. However, it is of interest to note that the axillary buds in plants treated with these agents developed into normal shoots. Further, long, white roots, which branched occasionally, appeared at the nodes and internodes below the treated regions. These roots generally formed in a single row on one side of the stem. These agents failed to induce tumors of any sort.

Control studies made on Bryophyllum and Kalanchoes treated with lanolin alone differed in that in the former species no reaction occurred. In the Kalanchoes the treatment with lanolin resulted in the development of a cluster of roots over the treated area. No tumors or nodular masses were observed. It appears from these experiments that the stimulus to root formation on the Kalanchoes treated with the carcinogenic agents is due to factors other than the applied chemicals. It appears evident that the adventitious roots are not the products of tumors. It seems possible that these roots may be the product of the host acted upon by some normal plant substance such as a wound hormone, and that the production of these roots may be independent of bacterial products[7] or of stimuli produced by tumor tissue induced by bacteria.[10]

Conclusions. Scharlach red, dissolved in ether and applied to decapitated shoots of *Kalanchoe Daigremontiana,* produces crown gall-like overgrowths which are characterized by leafy shoots and roots, and which resemble typical crown galls induced by *P. tumefaciens.*

Other carcinogenic agents as 1,2,5,6-dibenzanthracene, methylcholanthrene, and benzpyrene applied in lanolin cause injuries to the treated stem without inducing overgrowths on the Kalanchoe. Roots are produced by the Kalanchoes below and above the areas treated with 1,2,5,6-dibenzanthracene, methylcholanthrene, and benzpyrene. Indole acetic acid induces an abundance of long, white roots on the Kalanchoe, together wth small intumescences which are viable for only short periods. Roots are also produced on injured Kalanchoes when treated with lanolin alone. Root formation on the Kalanchoe induced by substances other than the heteroauxin studied here, results from injury which stimulates the host cells to produce root-forming substances. These substances appear to be transported to parts of the stem below and above the treated areas.

[10] Locke, S. B., Riker, A. J., and Duggar, B. M., *J. Agr. Res.,* 1938, **57**, 21.