RESEARCH NOTE

DEVELOPMENT OF WOUND TUMORS BY DIFFERENT VARIETIES AND LINES OF THE COMMON BEAN (PHASEOLUS VULGARIS L.)

Tumors (fig. 1) developed on wounds following the pruning of several bean plants (varieties Harvester and Jamaica), which had been inoculated with the mosaic virus of *Rhynchosia minima* (L.) DC. by means of the whitefly vector *Bemisia tabaci* Genn., during early 1969 while investigating the host range of the virus.

This virus is known to cause enations, malformations, and growth derangements in various plant hosts.[1,2,3] It was assumed to be the tumor-inducing factor at first because the tumors developed on virus-affected plants and because the control plants did not develop such tumors in our preliminary series of experiments.[4] This view became untenable, however, when in later experiments several tumors appeared on mosaic-free, control bean plants of the variety Diablo.

Extensive studies therefore were conducted under carefully controlled conditions from July 1969 until March 1970 in an effort to determine the cause or causes of these tumors. Simultaneous tests, involving hundreds of mosaic-affected and mosaic-free plants representing more than 70 bean varieties and lines, were performed in two separate greenhouses at the Agricultural Experiment Station of the University of Puerto Rico, Río Piedras, P.R. These tests were made to determine whether the presence of *Rhynchosia* mosaic virus in bean plants was *conditio sine qua non* for the development of wound tumors. The results clearly indicated that many varieties of bean plants could develop stem, pod, and petiolar tumors after wounding and in the absence of both *Rhynchosia* mosaic virus and whitefly vector. Bean plants of the varieties Criolla, Jamaica, Diablo, Dominicana, and Experimental No. 1208, as well as others, developed tumors fairly consistently between 10 and 14 days after being abraded, stabbed by insect pins, or lacerated. Following laceration, some plants developed chains of tumors. Others, as the one shown in figure 2, developed tumors at locations other than at the wounded area (insect pin no. 1). Routine microscopic

[1] Bird, J., A whitefly transmitted mosaic of *Jatropha gossypifolia*, Agr. Exp. Sta., Univ. P.R., Tech. Paper 22, 1957.

[2] Bird, J., Infectious chlorosis of *Sida carpinofolia* in Puerto Rico, Agr. Exp. Sta., Univ. P.R., Tech. Paper 26, 1958.

[3] Bird, J., A whitefly-transmitted mosaic of *Rhynchosia minima* and its relation to tobacco leaf curl and other virus diseases of plants in Puerto Rico, *Phytopathology* (Abstr.) *52*(3): 286, 1962.

[4] Findings from these preliminary studies were presented at the November 28, 1969 meetings of the Puerto Rico Chapter of the Phi Kappa Phi Society.

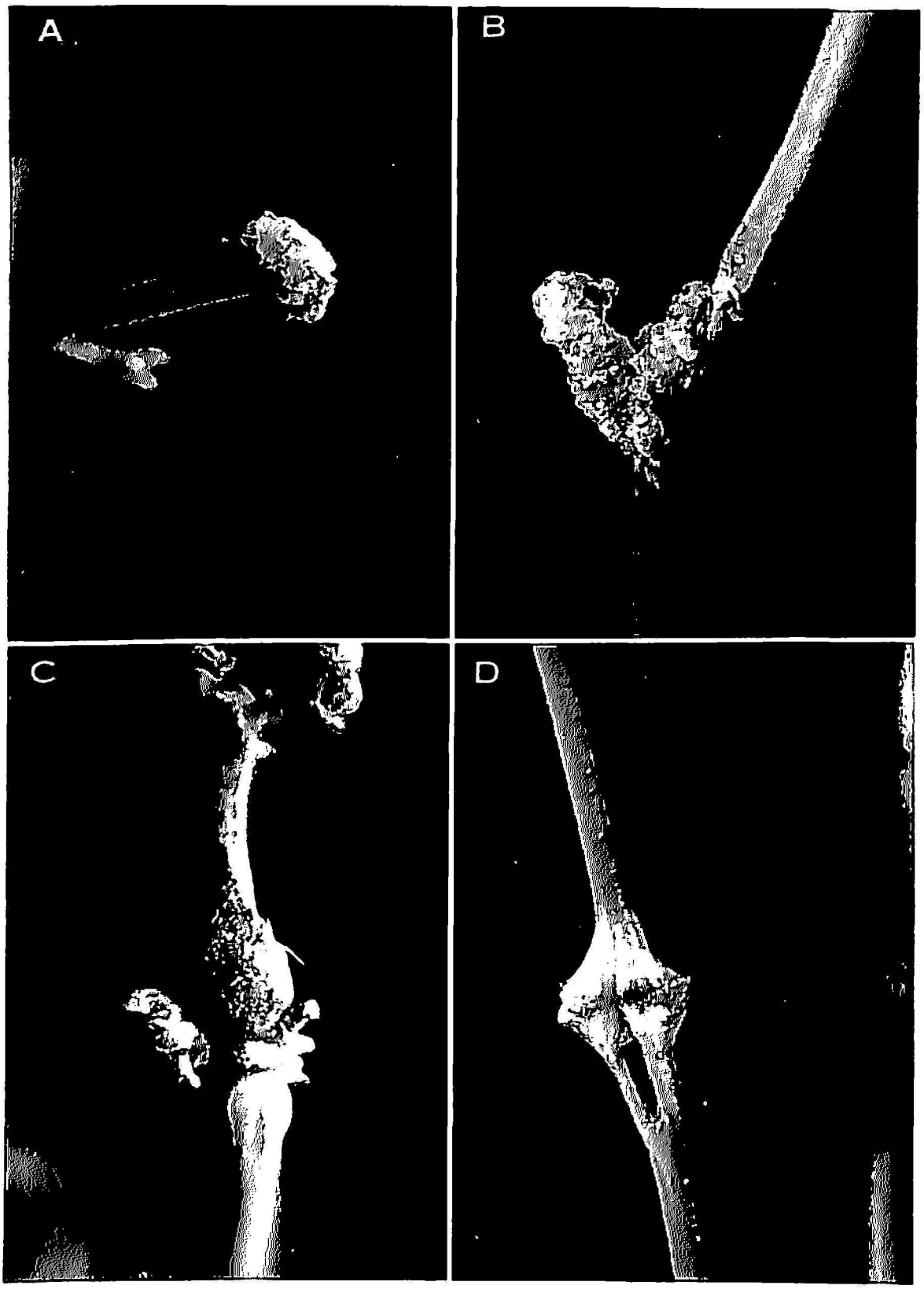

FIG. 1.—Wound tumors on bean plants produced by: A. Pruning, variety Jamaica. B. Knife-wound, variety Criolla. C. Stabbing (insect pin), variety Criolla. D. Abrasion and lodging, variety Jamaica.

examination of tumor tissue did not reveal the presence of bacteria in deeper tissues. Seed from neoplasm-prone varieties were surface disinfected by dipping for 2 minutes in a sodium hypochlorite solution (Clorox 1/10), subsequently washed in sterile distilled water, and transferred to deep germination plates containing sterile potato dextrose agar. After germination, aseptically-grown plants in the plates were stabbed at midstem with

FIG. 2.—Secondary tumor on a bean plant of the variety Criolla. Stem was stabbed with an insect pin below the first set of leaves but developed tumor some distance above the wounded area.

sterile insect pins. Two of 16 plants developed small tumors at the site of wounding (below the first set of leaves). One plant developed a small secondary tumor a few millimeters above the primary one. The results indicated that these abnormal growths were different from crown gall tumors, and that they were not initiated by fungi or bacteria introduced from outside.

Soil mixtures employed at the Agricultural Experiment Station usually are enriched with well-aged, composted, sugarcane filter-press cake. Such press cake is believed by some to contain growth-promoting and growth-modifying compounds. Because compounds of this type have been shown

capable of promoting plant cell proliferation, tests were made to determine whether the tumor-inducing factor resided in the soil mixture.[5,6] Plants of the tumor-prone variety Jamaica were grown in various substrates; i.e., washed sterile sand, pure filter-press cake, soil mix containing filter-press cake, and filter-press cake combined with sterile, washed sand. The results of these tests left no doubt that the plants developed tumors regardless of the substrate upon which they were grown, although the tumors were somewhat larger among plants grown on a substrate containing filter-press cake (fig. 3).

FIG. 3.—Large tumor on plant of the variety Jamaica. Plant was grown in cured sugarcane filter-press cake.

Because the response to wounding was reminiscent of the plant neoplasia induced by the wound tumor virus, a search was made for virus particles in tumor cells.[7,8] Fresh tumor tissues were cut into small pieces, approximately 1 $mm.^3$ in volume, and fixed in cold 3-percent glutaraldehyde in 0.2 M phosphate buffered solution at pH 7.3. Fixation was at 4° C. for 30 min-

[5] Black, L. M., and Lee, C. L., Interaction of growth regulating chemicals and tumefacient virus on plant cells, *Virology 3*(1): 146–59, 1957.

[6] Mitchell, J. W., and Preston, Wm. H., Jr., Secondary galls and other plant growth modifying effects induced by translocated α-methoxyphenylacetic acid, *Science 118* (3070): 518–19, 1953.

[7] Black, L. M., A virus tumor disease of plants, *Amer. J. Botany 32:* 408–15, 1945.

[8] Shikata, E., and Maramorosch, K., An electron microscope study of plant neoplasia induced by wound tumor virus, *J. Nat. Cancer Inst. 36:* 97–116, 1966.

utes, and postfixation in 2-percent osmium tetroxide in the phosphate buffered solution with 0.2 M sucrose at 4° C. for 2 hours. After rinsing in the buffered solution for a few minutes, the fixed materials were dehydrated in graded ethanol and 100-percent propylene oxide at 4° C. and embedded in an epoxy resin, Epon 812. Sections, approximately 50 mμ thick, were cut with diamond knives on a Porter-Blum MT-II microtome, stained with 8-percent magnesium uranyl acetate for 3 minutes at room temperature, and with 0.4-percent lead citrate for 3 minutes. The specimens were examined by Dr. H. Hirumi at the Boyce Thompson Institute in Yonkers, New York under a Siemens Elmiskop I electron microscope at 80 KV. No virus-like particles were detected in the tumor cells.

It is of interest to note that Howell and Kremer recently reported the occurrence of wound tumors in Pinto beans at Beltsville, Maryland.[9] It is possible that these authors may have observed the phenomenon reported herein.

Bean tumors provide a model for the subjective study of a dynamic problem of abnormal and autonomous cell growth and division of unknown cause. One can only speculate at this moment about the etiology of tumors such as these. It seems conceivable that wounded tissues release breakdown substances that stimulate cell proliferation. That such substances might be translocated is indicated by the tumors frequently developing at sites other than at the primary site of wounding. This reasoning would be consistent with the findings of Mitchell and Preston relative to the production of galls by translocated α-methoxyphenylacetic acid.[10] Other compounds related to 2,4-dichlorophenoxyacetic acid have been shown to induce formation of secondary galls in plants.[11]

A single dominant gene has been incriminated recently as the cause of certain neoplastic growths on the pods of peas by workers at the John Innes Institute, Norwich, England.[12] The quality of light falling on the pod was found to affect the activity of this gene.

The tumors under investigation resemble in some aspects the so-called Kostoff genetic tumors developing spontaneously in certain interspecific *Nicotiana* hybrids[13,14] These commonly arise at points of irritation and

[9] Howell, R. K., and Kremer, D. F., Tumor development in response to wounding tempo bean (*Phascolus vulgaris*), *Phytopathology*. (In press.)

[10] Mitchell, J. W., and Preston, Wm. H., Jr., *op cit.*, p. 591.

[11] Beal, J. M., Further observations on the telemorphic effects of certain growth-regulating substances, *Botanical Gaz. 106:* 165, 1944.

[12] Anonymous, Cancerous peas, *Nature 224:* 1245–46, 1969.

[13] Kehr, A. E., Genetic tumors in *Nicotiana*, *Am. Nat. 85:* 51–64, 1950.

[14] Kehr, A. E., and Smith, H. H., Genetic tumors in *Nicotiana* hybrids—Abnormal and Pathological Plant Growths, Brookhaven Symposia in Biology No. 6: 55–78. 1954.

all attempts have been unsuccessful to isolate a causative agent such as a fungus, a bacterium, or a virus. Skoog found these tumors depend upon the ratio of auxins and cytokinins in the plant tissue.[15] High auxin to cytokinin ratio leads to neoplastic growth. One therefore can speculate that bean varieties especially prone to tumor formation upon wounding might also have such a "hormonal imbalance" in their genetic constitution. This working hypothesis will be investigated as a possible mechanism of spontaneous bean tumor formation. The regulatory mechanisms controlling normal growth in the tumor-prone bean varieties seem to be so unstable that simple irritation is all that is needed to initiate and develop neoplasms. Thus, this tumor problem may well represent an extreme aspect of plant development.

The incorporation of foreign germ plasm may, in certain instances, bring about disturbances of the growth-regulatory mechanism with consequent interruption of the normal metabolic sequence at maturity and, at times, at the seedling stage according to Kehr and Smith.[16] According to these authors, this causes a change in growth patterns and finally results in abnormal, largely undifferentiated growth. The above thesis does not preclude the possibility of a pollen transmitted, seed-borne entity (a "naked" or "complete" virus) which might be incorporated with foreign germ plasm into a recipient variety. It is relevant at this point to mention that bean mosaic virus, which is seed transmitted, is also transmitted to healthy bean plants through the agency of the pollen of infected plants according to Reddick.[17]

The writers presently are screening many varieties and lines of beans in an effort to isolate a reliable, non-tumor-forming variety for transmissibility purposes.

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[15] Skoog, F., Growth and organ formation in tobacco tissue cultures, *Amer. J. Botany 31:* 19–24, 1944.

[16] Kehr, A. E., and Smith, H. H., *op. cit.* p. 592.

[17] Reddick, D., La transmission du virus de la mosaique du haricot par le pollen, Ext. du Deux. Congr. Int. Path. Comp. 363–66, 1931.

ERRATUM

Please note that figure 2, page 394 and figure 4, page 397, in the paper CYTOLOGY AND TAXONOMIC DESCRIPTION OF TWO *BRACHIARIAS* (CONGOGRASS AND TANNERGRASS), A. Sotomayor Ríos *et al.*, which appeared in J. Agr. Univ. P.R. *54* (2): 390–400, 1970, is in error:

The legend for Fig. 2 should read Congograss, while the legend for Fig. 4 should read Tannergrass.