Responses of Crown Gall Tissue to Gravity Compensation

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ABSTRACT

Increased weight of gravity-compensated crown gall tumors developing on carrot disks in comparison with those exposed to 1 *g* has been reported. An increase in radius of the meristematic rings of growth centers in these teratoma type galls was observed and provides an explanation for this phenomenon. The crown gall system is proving to be a sensitive and reliable indicator of the effects of simulated weightlessness on plant tissues.

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The crown gall system has been suggested for studies on gravitational responses of plant tissue (8). In this study, tumors were induced by inoculating cut surfaces of carrot disks with cells of *Agrobacterium tumefaciens* (E. F. Sm. & Towns.) Conn. Statistically significant larger tumors developed on surfaces facing the root apex than on those facing the base of the stem of such disks after incubation at 28 C for 21 days. This polarity effect was not altered when tumors were generated on clinostats; however, the fresh and dry weights of tumors were significantly larger when gravity compensated. This latter effect was interpreted as the result of an increase in metabolic activity in compensated tumors as demonstrated by Dedolph et al. (2). The same mechanism may operate in reducing the incubation period of rust on compensated bean plants (1).

In continuing studies we are reporting results of investigations initiated to determine the mechanisms responsible for tumor responses due to gravity compensation.

MATERIALS AND METHODS.—Carrots were cross-sectioned and one section was oriented as in the original root and the other inverted. These disks were suspended aseptically on a plastic support plate inserted between two halves of a petri dish as illustrated by Wells and Baker (8). Water agar (1.5%) was poured on the plastic support plate to maintain a high relative humidity, and to seal the suspended carrot sections. The upper surfaces of both sections were inoculated with bacterial suspensions (1×10^8 cells/ml) of *A. tumefaciens* strain B_6, grown in Stoner's glutamate medium. Each disk received 0.2 ml of suspension.

Two multiple unit "single axis" clinostats similar to the one illustrated and described by Gordon and Shen-Miller (3) were used to elicit gravitational responses. One clinostat was oriented so that the disks and developing tumors were oriented vertically with respect to gravity (hereafter identified as vertically-rotated) and the other was oriented to give horizontal placement and rotation of tissues (hereafter identified as horizontally-rotated or gravity-compensated). The speed of rotation in either case was 2 rpm. Two other treatments were used as nonrotated controls in all experiments. In one control, tumors were oriented vertically with respect to gravity, and the containers were placed on a table (hereafter identified as vertical-stationary); in the other, the containers were placed on their sides (hereafter identified as horizontal-stationary). There were six replications in each treatment group and experiments were repeated three times.

Tumors were generated on carrot disks for 21 days, excised and fixed with formalin-alcohol-acetic acid (FAA) or Craf III fixatives. They were dehydrated in an ethanol-xylene series and stained with safranin O-fast green, or Heidenhain's iron hematoxylin (7).

Following FAA fixation, the Krajcinovic amine reaction, confirmed by pectinase digestion, was used to specifically detect pectic acid in the cells of these tissues (5).

RESULTS.—Galls produced in all of the treatments were of the teratoma type (6); i.e., tumors with growth centers of small rapidly dividing cells developing among slowly dividing cells (Fig. 1). Vessel elements differentiating in the cores of the growth centers gradually resulted in disorganized xylem strands. Some cells outside the growth centers did not divide, but produced hypertrophied tissue composed of giant cells interspersed among smaller cells.

Tissues were studied to determine if there was an anatomical basis for the increase in weight and size of tumors observed when carrot disks were gravity compensated (8). The hypothesis that large tumors resulted from an increased frequency of growth centers per unit area was examined; however, an average of five to six growth centers per 4 mm^2 was observed in all sections regardless of treatment.

The most obvious difference in tumors was in the thickness of the meristematic ring surrounding the disorganized xylem tissue. Cells in the meristematic region were characterized by lack of intercellular spaces being derived from periclinal divisions which resulted in rows of cells oriented parallel with the surface of the gall. Measurements were made of the thickness of these meristematic tissues in units of cell numbers counted through the radius (thickness) of a meristematic ring as viewed in cross-section. Counts were made for 23-30 growth centers in each treatment and data were subjected to statistical analysis using Student's *t*-test.

Fig. 2 illustrates typical cross-sections of the growth centers observed following the various gravity

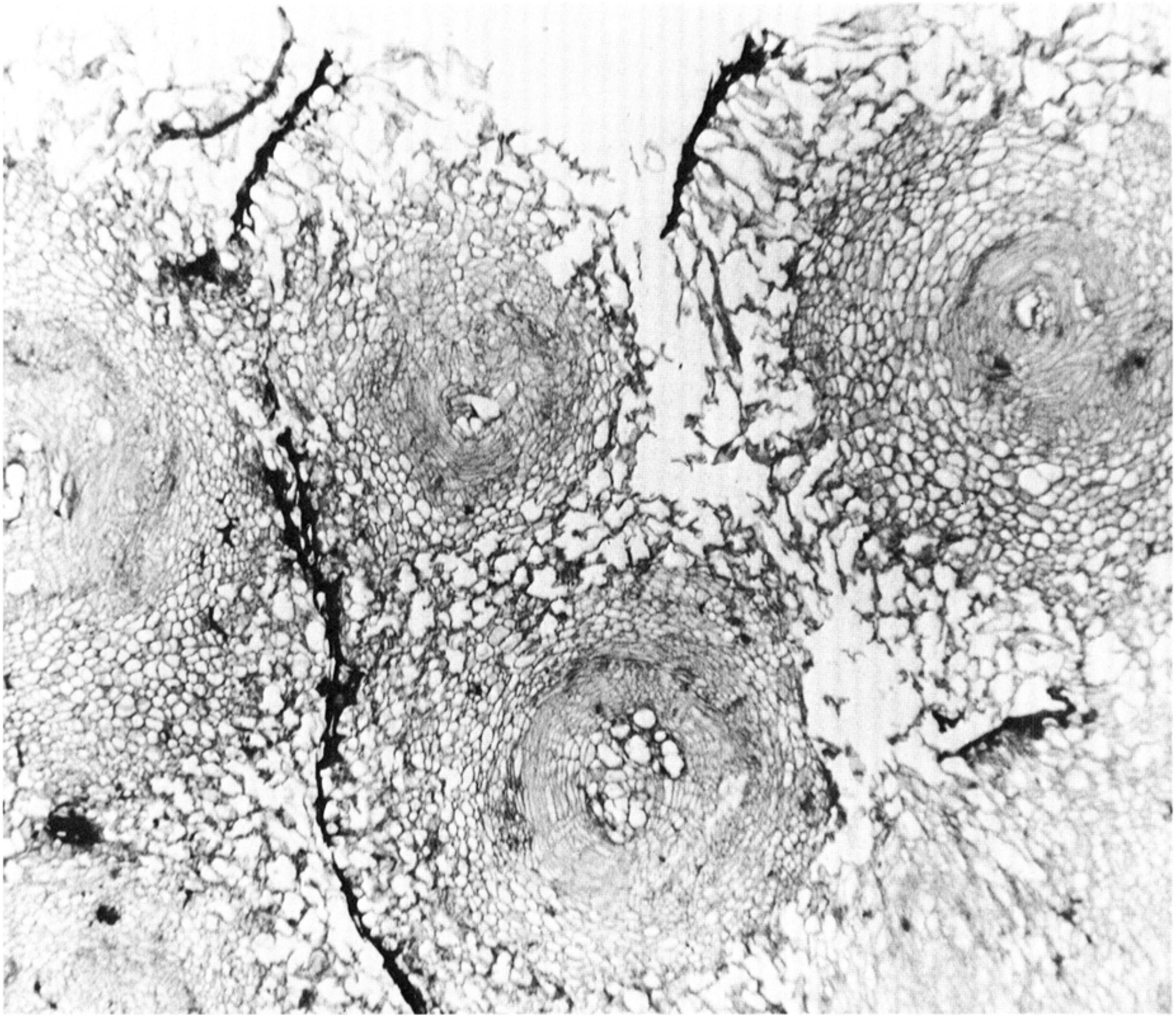

Fig. 1. Cross section of tumor incited by *Agrobacterium tumefaciens* on a carrot disk with growth centers of small rapidly dividing cells (× 13).

treatments, and Fig. 3 illustrates the data in graphic form. There was a highly significant difference ($P < 0.01$) between the number of cells in the radius of the meristematic ring in gravity compensated tissue (horizontally rotated) and the number in any of the noncompensated controls. The thickness in compensated tissues was an average of 9.4 cells per radius, while in noncompensated tissues it was 5.6 to 6.9 cells per radius. There was no apparent difference in size of individual meristematic cells among any of the treatments.

Attempts were made to determine whether xylem tissues in tumors were more disorganized as a result of being compensated. Xylem vessel elements were counted cross-sections on upper portions of tumors that appeared to be oriented so that no spiral thickenings were apparent (i.e., oriented approximately parallel to the positions of xylem in the original carrot disk host dissue). These numbers were compared with vessel elements in which wall sculpturing was apparent and thus not oriented parallel to vessel elements in the host tissue. The ratios of oriented to nonoriented xylem vessel elements averaged 1.2 for vertical-stationary, 1.1 horizontal-stationary, 1.4 vertical-rotated, and 1.3 for horizontal-rotated treatments, based on 19 to 28 observations in each treatment. Thus, there was no difference in xylem organization due to compensation. Cross-sections of tumor tissues deeper into the gall, approaching the nonaffected host tissue, showed more normal orientation of xylem vessels. The reason for this became apparent when longitudinal sections were studied (Fig. 4). When the initial reaction to wounding and inoculation occurred, cells divided and enlarged parallel to the would surface. As the gall proliferated, however, disorganization became more pronounced in the outer portions of the tumor.

A typical Krajcinovic amine reaction (5) is illustrated in Fig. 5. In both compensated and noncompensated tumors differential deposition of pectic acid was detected. This correlated with the basic anatomy of each growth center in that the heaviest deposition was seen in cell walls comprising the xylem vessel element core (X), moderate

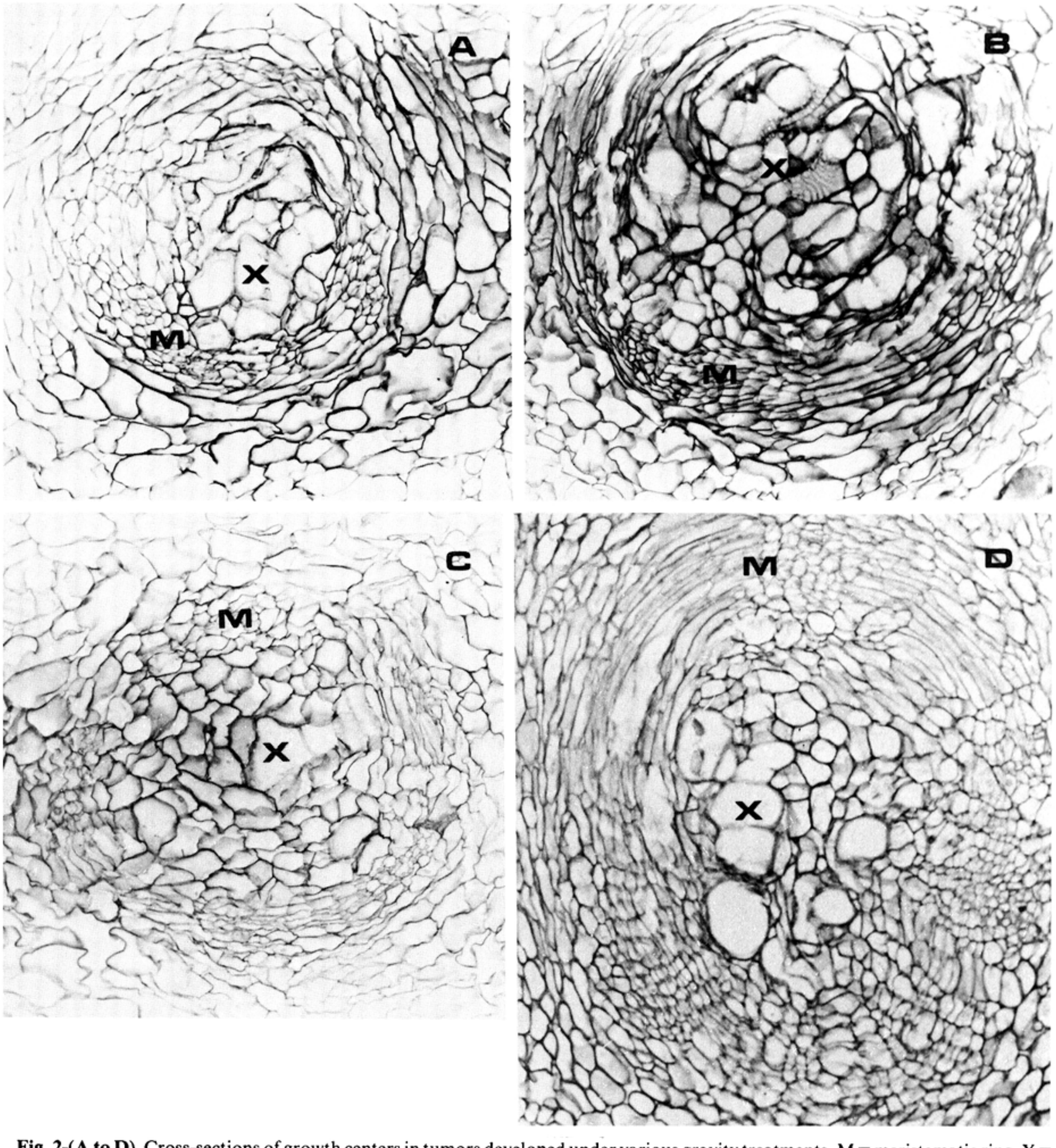

Fig. 2-(A to D). Cross-sections of growth centers in tumors developed under various gravity treatments. M = meristematic ring, X = disorganized xylem tissue (A-C, × 378; D, × 289). **A)** vertical-stationary. **B)** horizontal-stationary. **C)** vertical-rotated. **D)** horizontal-rotated or gravity-compensated.

deposition in the surrounding parenchyma (P), and especially low in the cell walls in meristematic ring (M).

DISCUSSION.—The increase in radius of meristematic rings of the growth centers in gravity-compensated crown gall tumor tissue provides an explanation for the increased weight of such tumors over noncompensated controls observed by Wells and Baker (8). This increase in radius together with the paucity of pectic compounds in the meristematic zone may be indicative of either hyperplasticity or delayed differentiation in these cells.

Since the teratoma type of tumor is disorganized, considerable variation in the tissues is expected. Xylem vessel elements in tumors just above the cut and inoculated surface of the host tissue were oriented with their long axes parallel to the elements found in the normal host tissue, however, as illustrated in Fig. 4. Thus, cross sections obtained in this area were not as disorganized and not as variable as those taken from tissue deeper in the tumor. Again, in compensated material, the radius of the meristematic ring in many cases was not greater than noncompensated controls in this region of the tumor. But, as the tumor tissue developed, more disorientation was observed in the xylem vessel elements, and the radius of the meristematic zone was typically greater in gravity-compensated tissue than controls. Variation in the size of these radii, however, was not excessive as indicated by the degree of standard error illustrated in Fig. 3.

It has been suggested (8) that the increased weight of gravity-compensated tumors results from enhancement of metabolism. Dedolph et al. (2) ascribed such enhancement to the more uniform orientation of metabolically active particulates in gravity-compensated cells leading to steeper solute concentration gradients between the particles and the adjacent cytoplasm.

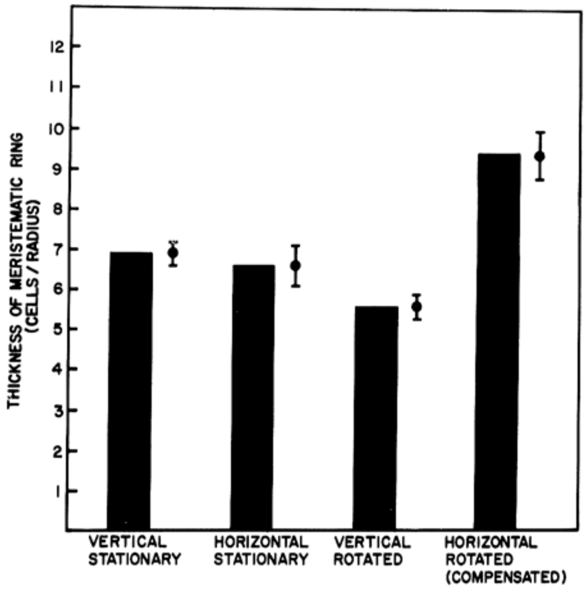

Fig. 3. Number of cells in radius of meristematic rings of growth centers in tumors incited by *Agrobacterium tumefaciens* on carrot disks following various gravity treatments. The standard errors shown as vertical brackets were computed from original data.

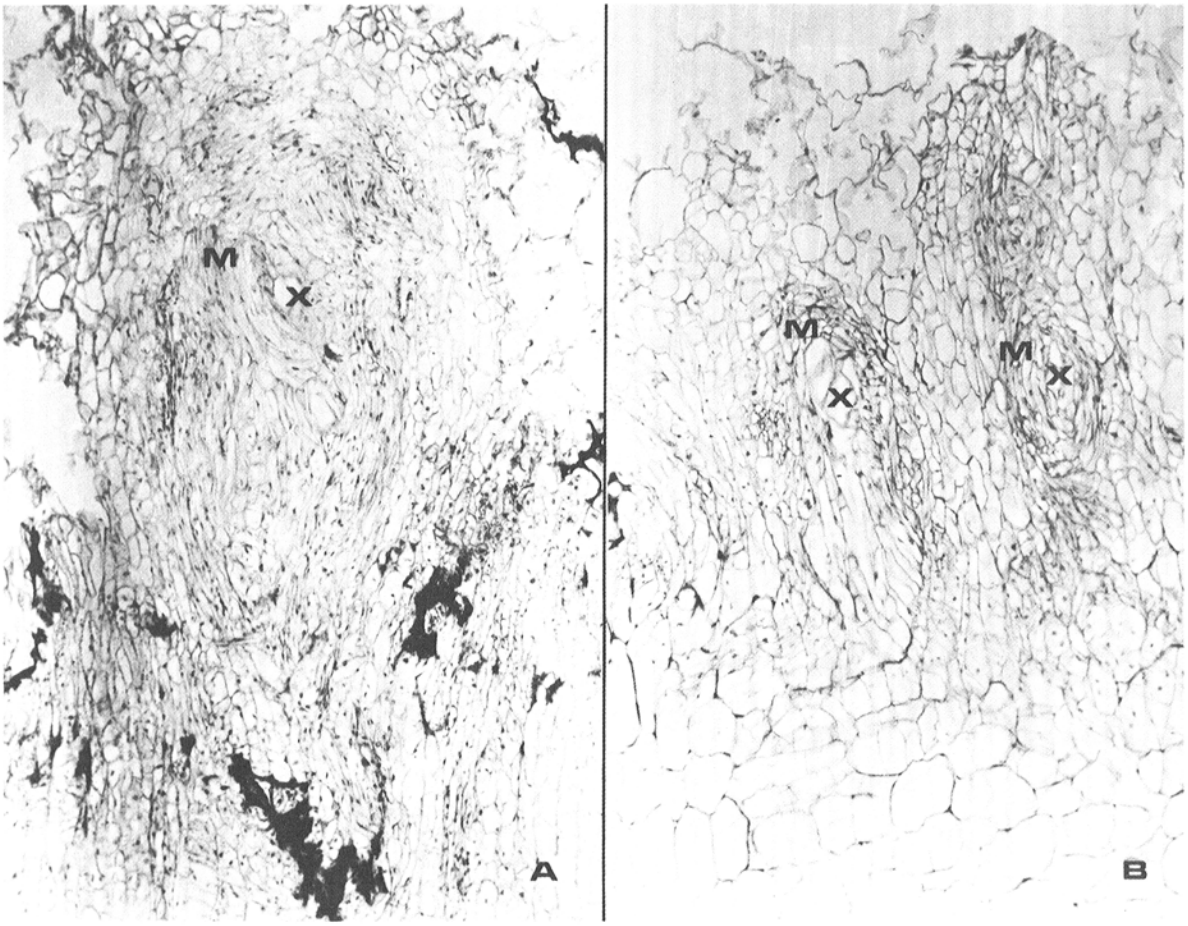

Fig. 4-(A, B). Longitudinal median sections of growth centers in tumors caused by *Agrobacterium tumefaciens* on a carrot disk developed under various gravity treatments. M = meristematic ring, X = disorganized xylem tissue (× 152). **A)** horizontally-rotated or gravity-compensated. **B)** vertical-stationary.

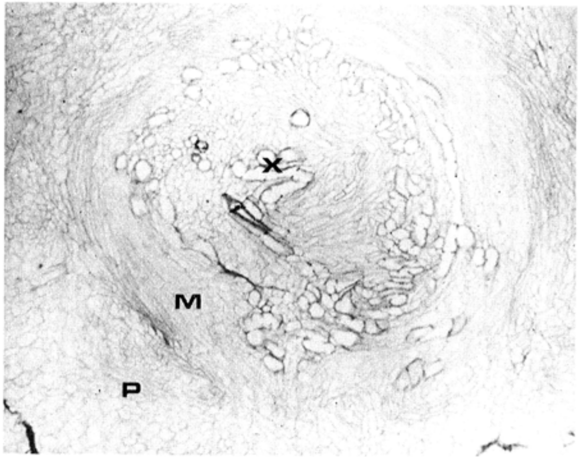

Fig. 5. Cross section of horizontal-rotated or gravity-compensated growth center. M = meristematic ring; X = disorganized xylem; P = parenchyma; Krajcinovic reaction (× 123).

Gordon and Shen-Miller (3) reviewed this hypothesis, reserving judgment until unknown effects of reorientation shear (due to sagging of shoots on the clinostat during rotation) are known. Since the carrot root and crown gall tumors developed in our studies are rigid structures, our results suggest that stress phenomena associated with rotation are not entirely responsible for enhanced metabolism.

The crown gall system herein described is proving to be a sensitive and reliable indicator of the effects of simulated weightlessness on plant tissues (4). Moreover, if comparisons of tissues developed in the weightless condition of satellites with those developed on clinostats are contemplated, the system has additional technological advantages. Experiments can be set up and then require no further attention for long periods of time. There is no instrumentation, the only requirement being that of maintaining an environment conducive to the generation of tumors. Since metabolism is supported by storage materials in the carrot disk, there is no power requirement for light. Thus, the crown gall system should provide a sensitive, but relatively inexpensive, means for determining whether responses of plant tissue in simulated weightlessness on earth is similar to those observed in the space environment.

LITERATURE CITED

1. CURTIS, C. R. 1967. Bean rust development in relation to gravity. Phytopathology 57:1025-1027.
2. DEDOLPH, R. R., D. A. OEMICK, B. R. WILSON, and G. R. SMITH. 1967. Causal basis of gravity stimulus nullification by clinostat rotation. Plant Physiol. 42:1373-1383.
3. GORDON, S. A., and J. SHEN-MILLER. 1971. Simulated weightlessness studies by compensation. Pages 415-426 *in* S. A. Gordon and M. J. Cohen (ed.), Gravity and the organism. The University of Chicago Press. 473 p.
4. HANCHEY, P., B. L. BAKER, and R. BAKER. 1975. Isozyme patterns in gravity compensated crown gall tissue. Phytopathology 65: (In press).
5. KRAJCINOVIC, V. M., V. PURIC, and M. KRAJCINOVIC. 1954/55. Der qualitative nachweis des Pektins in der Pflanzenzelle mit Hilfe der Aminreaktion. Acta Histochem. 1:76-81.
6. KUPELA, S. 1963. Crown gall as an anatomical and cytological problem: a review. Cancer Res. 23:497-509.
7. SASS, J. E. 1958. Botanical microtechnique. 3rd ed. Iowa State College Press, Ames. 228 p.
8. WELLS, T. R., and R. BAKER. 1969. Gravity compensation and crown gall development. Nature 233:734-735.