ENDOGENOUS ROOT-PROMOTING AND ROOT-INHIBITING FACTORS IN PEAR CUTTINGS IN RELATION TO BUD ACTIVITY

M. S. FADL[1] AND H. T. HARTMANN
Department of Pomology
University of California
Davis, California

In studies of methods of propagating rootstocks known to be resistant to "pear decline", it was noted that hardwood cuttings of 'Old Home' pear rooted readily whereas such cuttings of 'Bartlett' were very difficult to root (3). It was determined in these tests that nursery trees of own-rooted 'Old Home' could be propagated easily by taking hardwood cuttings in the fall, treating them with indolebutyric acid (IBA), then storing them for about 3 weeks in damp peat moss at 70°F. for roots to become just visible before planting in the nursery. 'Bartlett' pear cuttings, however, if handled in this manner formed no roots. But if 'Bartlett' cuttings, after receiving the IBA treatment, were placed out-of-doors (in late fall or early winter) upright in damp peat moss over bottom heat (80°F.) but with the top buds exposed to the natural winter-chilling (40 to 50°F), roots would form in about 3 weeks. Cuttings with such incipient rooting, if transferred to the nursery row, would then develop into vigorous nursery trees by the end of the summer.

In the present study it was planned to determine, if possible, what differences existed between 'Bartlett' and 'Old Home' enabling hardwood cuttings of the latter to root so

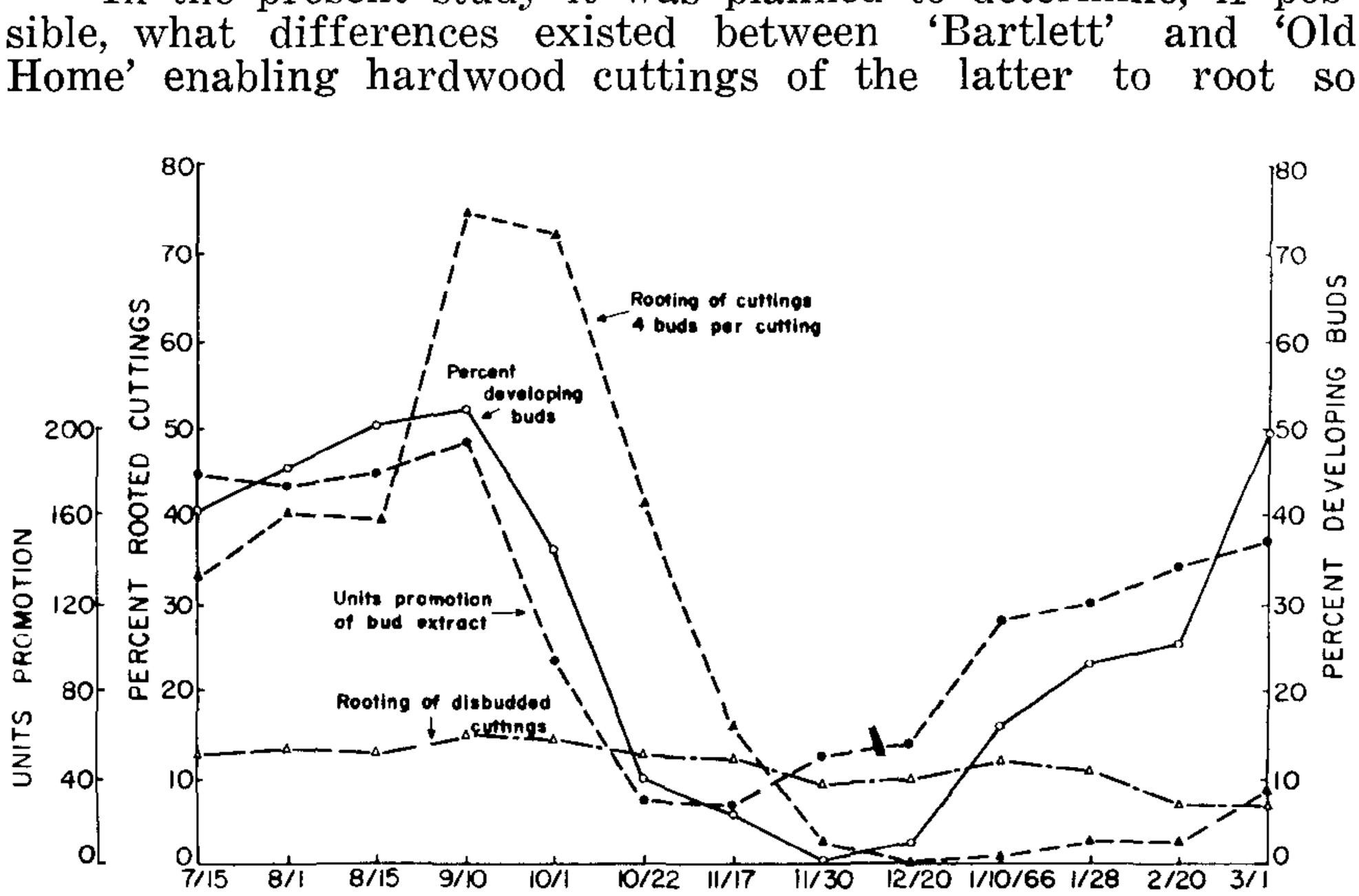


Figure 1 Effect of collection date on: rooting of 'Old Home' hardwood cuttings with 0 to 4 buds each, the percent of developing (sprouting) buds, and the "units promotion" of bud extracts. The extracts were bioassayed by the mung bean rooting test for calculation of the "units promotion" values

[1]Present address Egyptian Agricultural Organization, Cairo, Egypt

much more readily, why chilling the buds of 'Bartlett' seemed to stimulate rooting, and why fall or early winter was an optimum time for taking the cuttings.

Figure 1 shows the changes in rooting obtained with 'Old Home' hardwood cuttings during the period from mid-July to the end of February and compares rooting of disbudded cuttings with those having 4-buds each. Also shown is the relationship between rooting and the potential physiological activity of the buds; that is, their ability to sprout when placed under optimum environmental conditions (2). Figure 2 (above) shows the effect of bud number on each cutting on the percent of cuttings rooting at different times of the year from October 1 to January 10 (2). It is apparent from these figures that with 'Old Home', the presence of buds stimulates rooting in mid-September when the buds are physiologically active but as the buds become deeper in the "rest" in midwinter, their presence inhibits rooting.

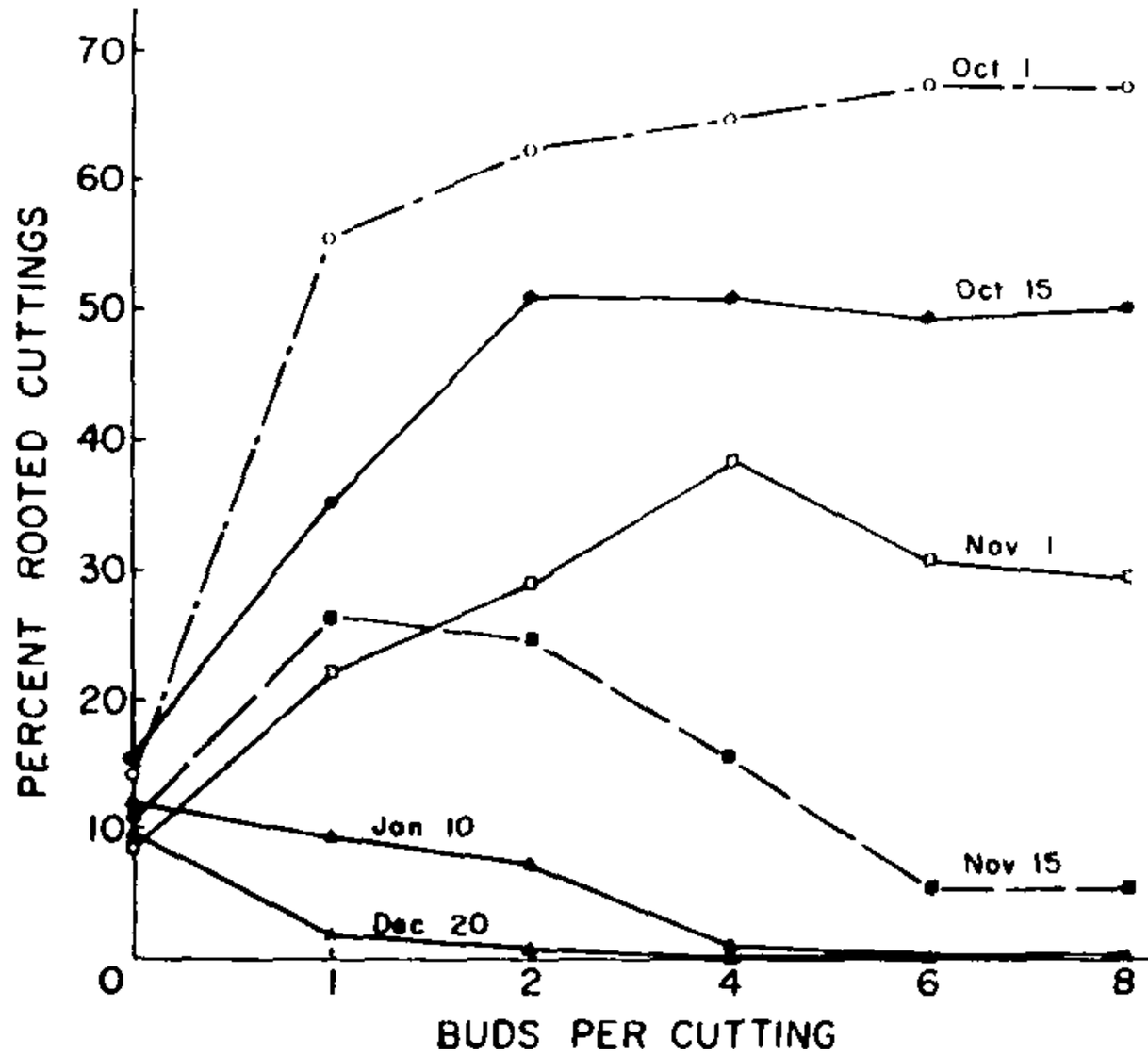
With 'Bartlett' cuttings, on the other hand, (Fig. 2 - center), rooting is consistently low and the presence of any buds completely inhibits rooting. However, if 'Bartlett' cuttings are placed vertically over bottom heat with the top buds exposed to chilling (45°F), the picture is completely changed. Rooting is increased considerably and, except for the January and February collection dates, the presence of buds is not associated with poor rooting (Fig. 2 - below).

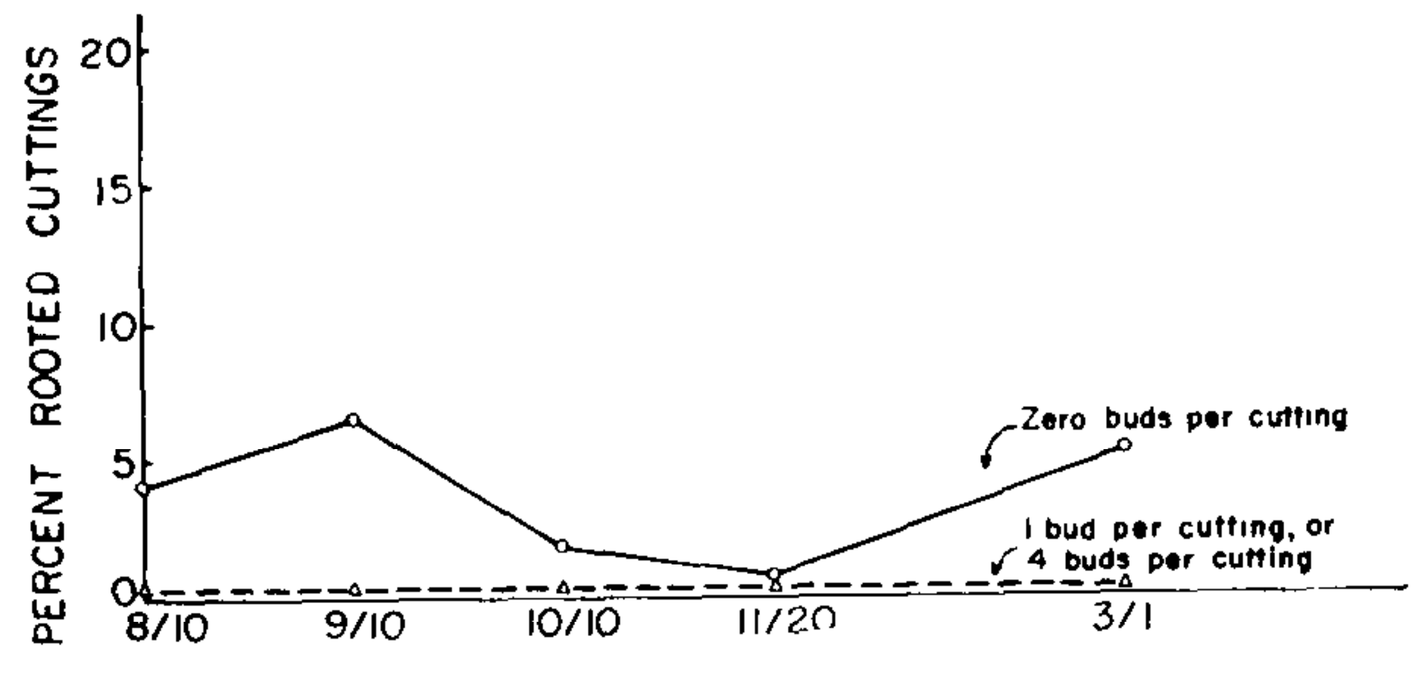
To study possible changes in the levels of naturally-occurring rooting promoters and inhibitors, or both, in the bases of the cuttings during the periods of active and inactive rooting, the mung bean bioassay as developed by Hess (4) was used after separation of the growth-active substances by paper chromatography.

'Old Home' and 'Bartlett' hardwood cuttings, about 20 cm long, were taken on September 10, 1965, from shoots produced that growing season. Samples, each consisting of ten 2.5 cm basal segments, were prepared from cuttings of both varieties as follows: (1) Cuttings taken immediately after collection from the trees (zero time). (2) Cuttings which had been stored in the rooting medium (moist peat moss) at 76°F for 24 hrs following treatment with 2000 ppm indolebutyric acid (IBA) in 50% (v/v) ethanol by the concentrated solution-dip method. (3) Cuttings which had been disbudded, (without IBA treatment) then stored in the rooting medium for 10 days. (4) Same as 3, but cuttings treated with 2000 ppm IBA. (5) and (6) Same as 3 and 4, respectively, except cuttings were stored in the rooting medium for 20 days.

Treatments 7, 8, 9 and 10 correspond to treatments 3, 4, 5 and 6, respectively, except that all cuttings had 4 buds each.

The excised basal segments of the cuttings were extracted with ethanol. For analysis, the extracts were concentrated to a final volume of 4 ml. Growth-active substances were separated by paper chromatography.





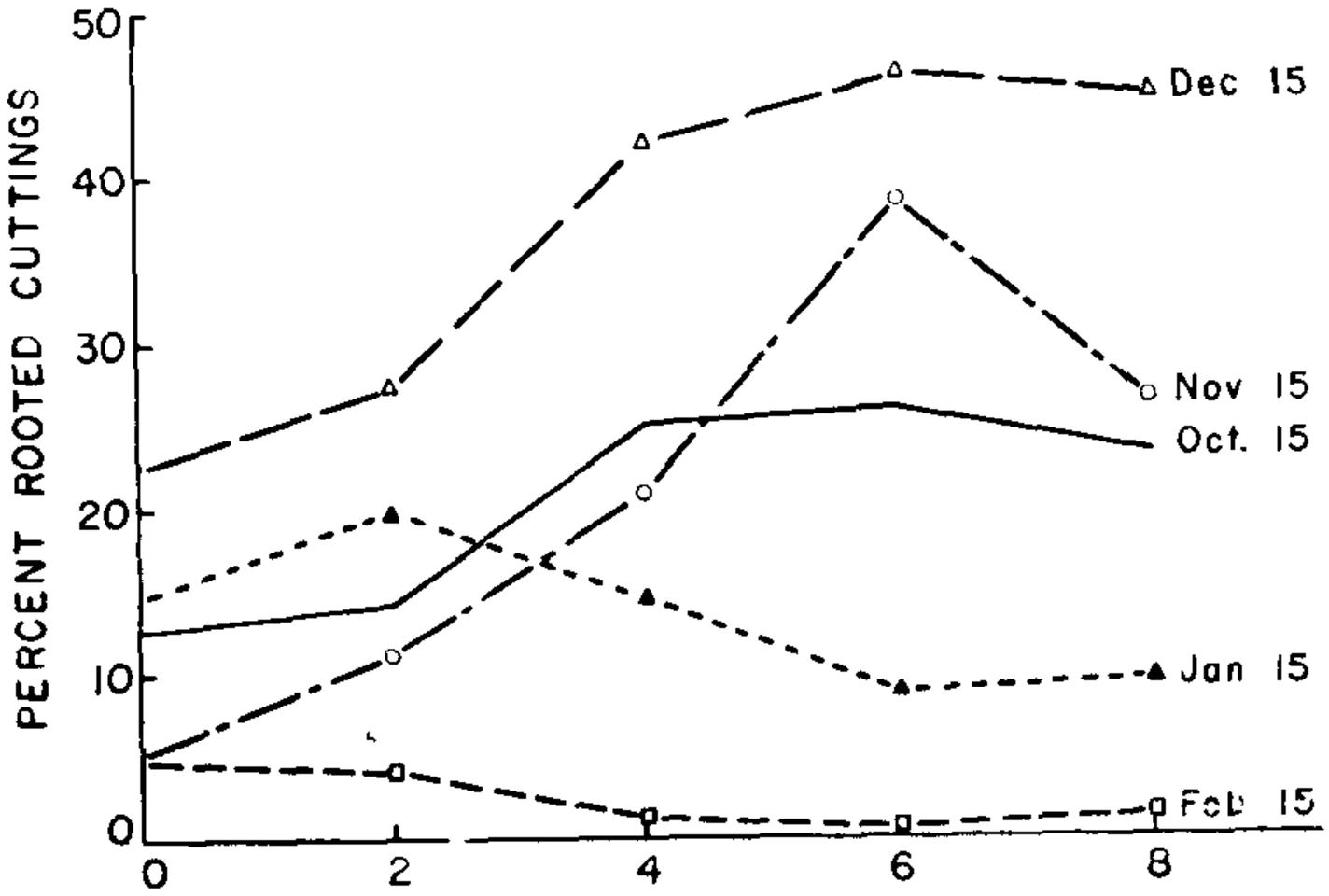


Figure 2. Relationship between bud number and rooting of hardwood pear cuttings. *Above*: 'Old Home' pear. *Center*: 'Bartlett' pear. *Below*: 'Bartlett' pear cuttings rooted over bottom heat with simultaneous chilling of top buds.

Paper Chromatography. A volume representing 250 mg dry weight of the extracted basal tissues was strip-loaded on 46.3 x 19.0 cm Whatman No. 3 MM chromatography paper. All chromatography was of the descending type, using isopropanol-water (10:1, v/v) as the solvent system. The chromatograms were developed uni-directionally for 14 hours until the solvent front was about 30 cm from the origin. The dried chromatograms were cut into 15 transverse strips of equal width; each strip was subsequently bioassayed by the mung bean rooting test. The paper strips above the origin served as controls. The chromatogram strips were transferred to 6.8 x 2.0 cm shell vials into which 10 ml of distilled water was added. An equilibration period of approximately 6 hours was allowed before mung bean cuttings were added.

Mung Bean Bioassay: Mung bean seeds were immersed for 5 minutes in a solution of 1 part Clorox (commercial hypochlorite) to 10 parts water, rinsed, and soaked in running tap water for 24 hours. Seeds were then planted and germinated. Five mung bean cuttings were placed in each vial, which also contained the chromatogram section. Distilled water was added daily to maintain the original water level throughout the rooting period. The number of roots on each cutting was counted after 6 days and the average number of roots per cutting was determined.

The results from the mung bean bioassays are shown in Figures 3, 4, 5, 6 and 7. In the histograms each column represents a chromatogram section, the origin being on the left. Those columns above the horizontal line (control) indicate promotion of rooting; columns below the line indicate inhibition of rooting. The promotion or inhibition obtained from each chromatogram strip was due entirely to material(s) present on that strip, since there were no external additives in the vials other than distilled water.

Mung bean bioassay of 'Old Home' pear extracts obtained at zero time (prior to IBA treatments) showed a zone of promotion at chromatogram strips 12, 13, and 14. 'Bartlett' extracts showed considerably less promotion in the same zones (Fig. 3, a and b). 'Old Home' extract obtained from the cuttings 24 hours after they had been treated with an IBA solution (2000 ppm), however, showed considerably increased promotion at the same zone (strips 12 to 14) while 'Bartlett' extract did not show as much increase (Fig. 3, c and d).

The rooting activity of extracts obtained from basal segments of *disbudded* cuttings, both treated and untreated with IBA, followed by storage in the rooting medium for 10 days, is given in Figure 4. Extracts of cuttings not treated with IBA (Fig. 4, a and b) showed much less promotion activity than did those obtained from IBA-treated cuttings (Fig. 4, c and d). Furthermore, extracts of budless 'Old Home' cuttings had less inhibitory and more promotion activity at strips 12 to 14 than did those of 'Bartlett' but, in both cases, the amount

of promotion was relatively low. 'Old Home' cuttings prepared with such treatments (no buds and no IBA) root very poorly. On the other hand, 'Old Home' cuttings with 4 buds each, if treated with IBA, show very high rooting percentages, especially when taken in September (Fig. 1).

The activity of extracts obtained from similar *disbudded* cuttings, either treated or not treated with IBA, then stored

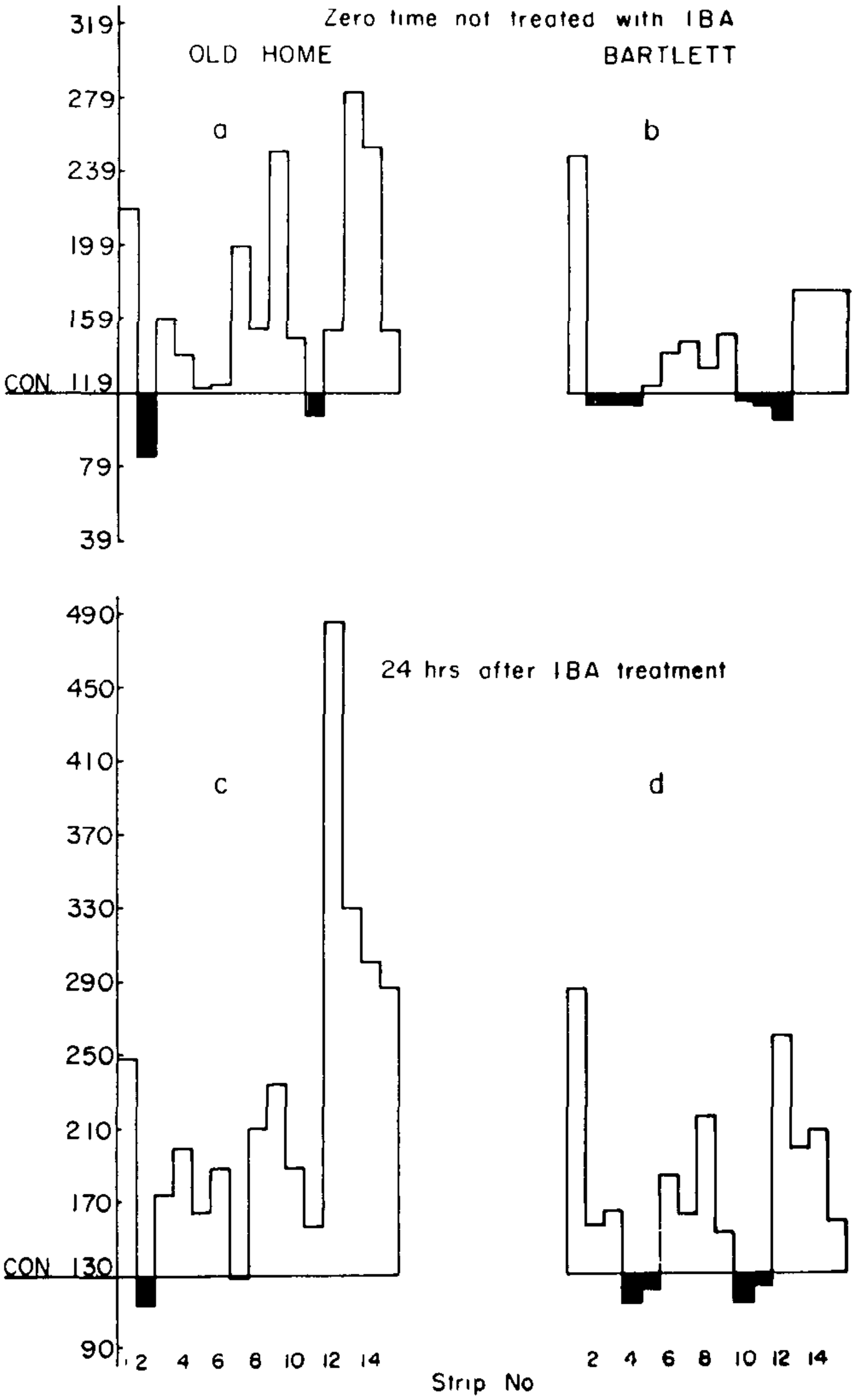


Figure 3 Histograms showing the root-promoting or inhibiting activity of diffusates from chromatogram strips tested by the mung bean bioassay Cuttings sampled on September 10 and stored in moist peat moss at 70°F CON = control, above control = promotion; below = inhibition Each chromatogram represents 0 25 gm of basal segments of cuttings.

in the rooting medium for a longer period (20 days) is shown in Figure 6. Extracts of disbudded cuttings, not treated with IBA, showed no inhibitory activity and, in the case of 'Bartlett' extracts, showed a considerable zone of promotion at strips 6, 7, and 8; a similar promotion effect is almost absent from 'Old Home' extract. It was noted previously that disbudded 'Bartlett' cuttings when placed to root, callus very heavily, and give about 6% rooting. Disbudded 'Old Home' cuttings, on the other hand, show a very low rooting percentage, if they root at all, and the basal tissues of most cuttings die during a period of about 25 days; this is the same behav-

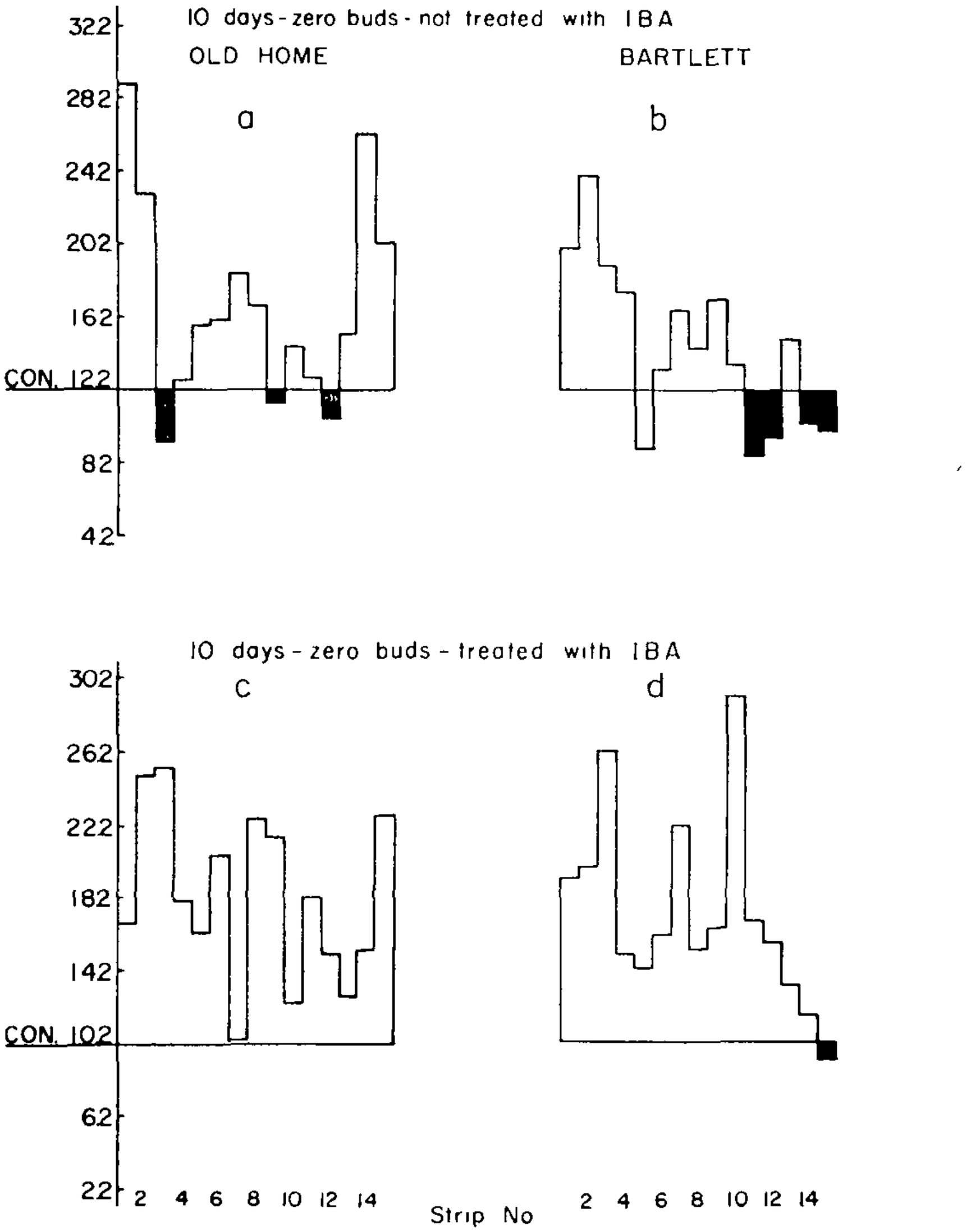


Figure 4 Histograms showing the root promoting or inhibiting activity of diffusates from chromatogram strips tested by the mung bean bioassay. Cuttings sampled on September 10 and stored in moist peat moss at 70°F for 10 days. Zero buds per cutting. Each chromatogram represents 0.25 gm of basal segments of cuttings

ior shown by 'Bartlett' cuttings which have buds and are treated with IBA.

In the mung bean bioassay only slight root promotion activity, but considerable inhibition activity, appeared in basal extracts from *disbudded* and IBA-treated 'Old Home' cuttings (Fig. 6, c). Root promoting activity in the bioassay with extracts of disbudded 'Bartlett' cuttings, at strips 12 to 14, was also low, whether or not the cuttings were treated with IBA (Fig. 6, b and d).

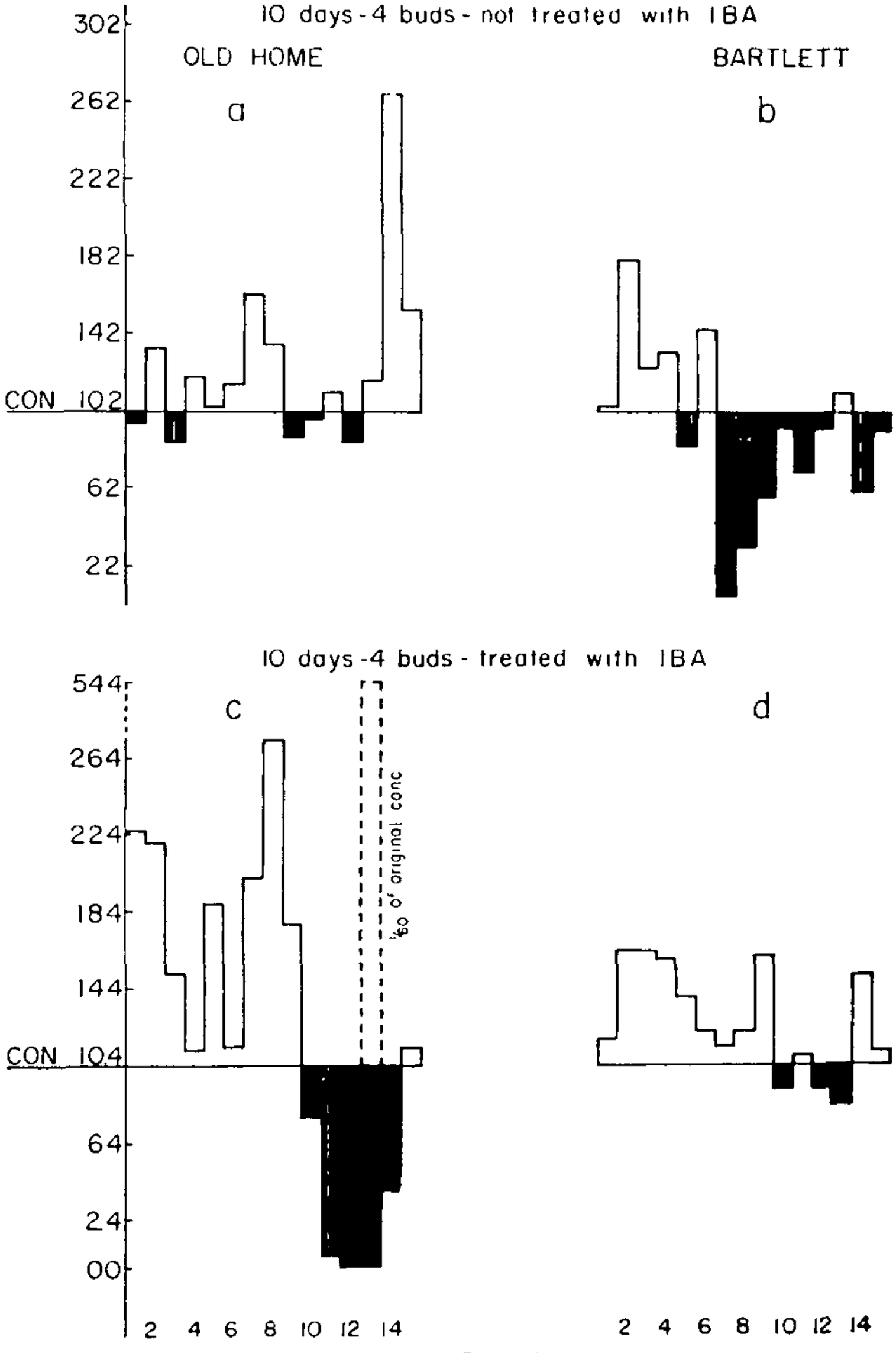


Figure 5 Histograms showing the root promoting or inhibiting activity of diffusates from chromatogram strips tested by the mung bean bioassay Cuttings sampled on September 10 and stored in moist peat moss at 70°F for 10 days Four buds per cutting Each chromatogram represents 0 25 gm of basal segments of cuttings

The biological activity in the mung bean test of basal extracts obtained from *4-budded* cuttings and stored in the rooting medium for 10 days is shown in Figure 5. If the cuttings were not treated with IBA a considerable amount of inhibitory activity was obtained from 'Bartlett' extracts (Fig. 5,

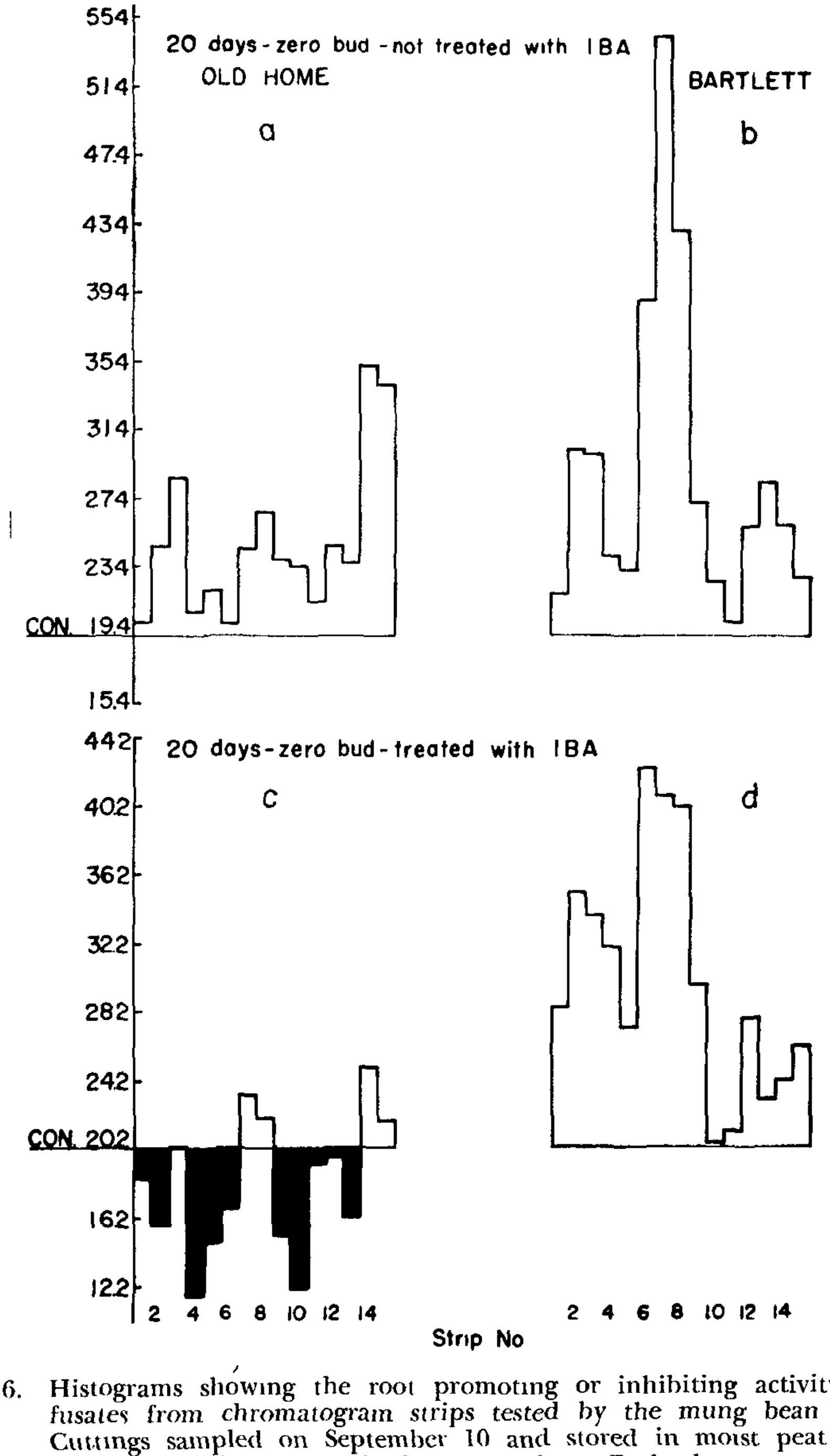


Figure 6. Histograms showing the root promoting or inhibiting activity of diffusates from chromatogram strips tested by the mung bean bioassay Cuttings sampled on September 10 and stored in moist peat moss at 70°F for 20 days Zero buds per cutting. Each chromatogram represents 0 25 gm of basal segments of cuttings.

b) but 'Old Home' extracts (Fig. 5, a), showed less inhibition.

Basal extracts obtained from 'Old Home' cuttings which had buds and were treated with IBA were expected to show a large amount of promotion, especially at strips 12 to 14, since cuttings treated this way and placed for rooting at this time of the year gave the highest rooting percentage obtained. The

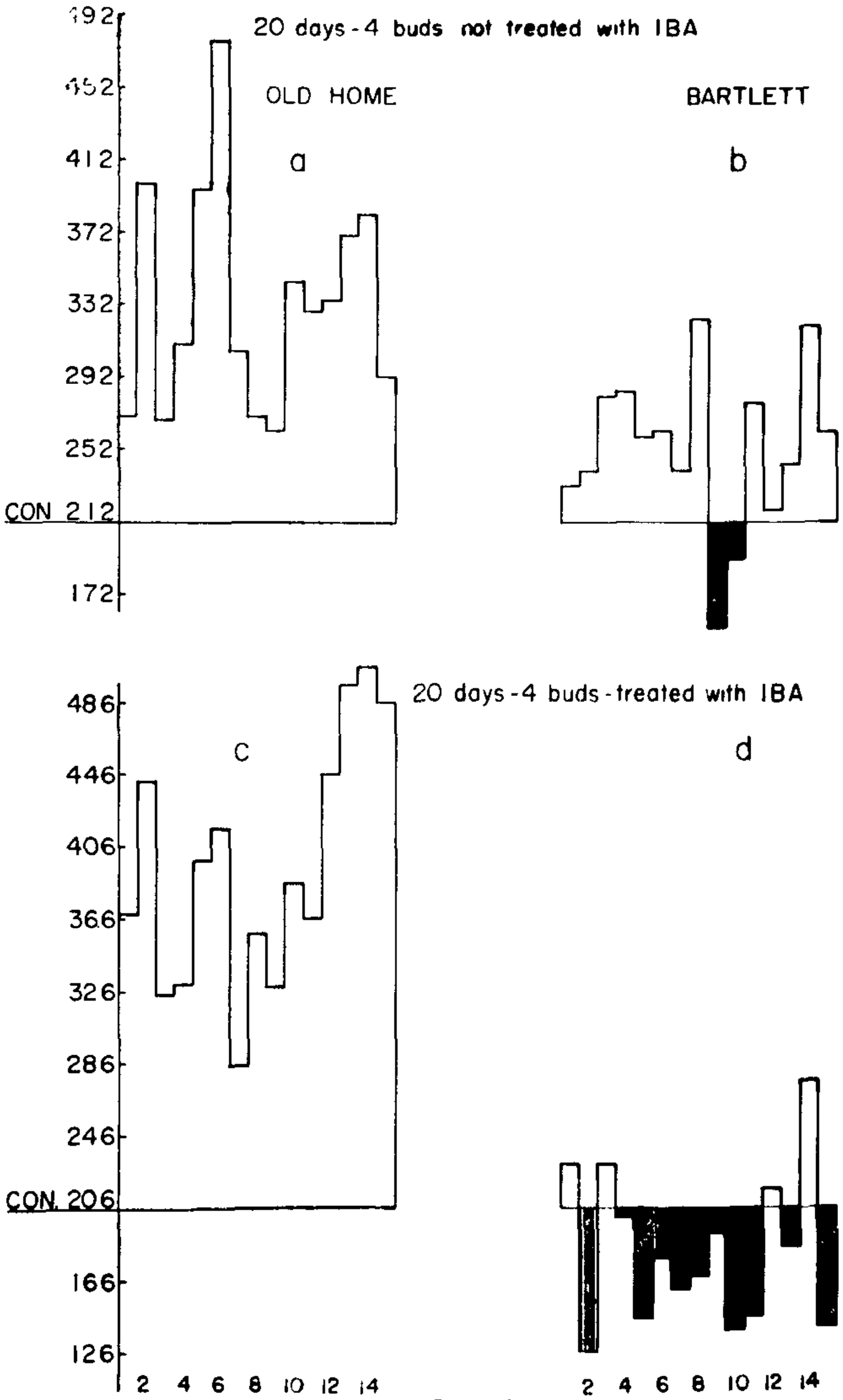


Figure 7 Histograms showing the root promoting or inhibiting activity of diffusates from chromatogram strips tested by the mung bean bioassay Cuttings sampled on September 10 and stored in moist peat moss at 70°F for 20 days Four buds per cutting Each chromatogram represents 0 25 gm of basal segments of cuttings

results shown in Figure 3, c support this prediction. However, rather than getting strong promotion where it was expected (strips 12-14), very definite inhitory activity appeared (Fig. 5, c). It was further noted that the mung bean cuttings used in the bioassay with these chromatogram strips not only did not root but showed a definite dark purple coloration in the veins of the 2 leaves. Since this was a rather difficult phenomenon to explain, it was decided to run a mung bean bioassay on a series of dilutions of the eluate obtained at these particular chromatogram strips, as well as on the remaining strips. As shown by the dotted bar in Figure 5, c, when an eluate from strip 13, where the inhibitory effect was most obvious, was diluted to 1/60 of its original concentration and applied to mung bean cuttings, considerable root promotion was obtained. The average number of roots was 54.4 per cutting in comparison with 10.4 for the control (Fig. 5, c). When the other strips (1-9) were tested with mung bean cuttings, however, a gradual decrease in root promotion took place as a result of the dilution.

The activity in the mung bean bioassay of extracts obtained from the bases of *4-budded* 'Old Home' and 'Bartlett' cuttings held in the rooting medium for 20 days (rather than 10 days), both treated and untreated with IBA, is shown in Figure 7. The activity of 'Old Home' extracts, especially those obtained from IBA-treated cuttings, at strips 12 to 14, showed about the same behavior as did the diluted eluates shown in Figure 5, c. There was a large zone of promotion at these strips, but the intense purple coloration noted earlier (after 10 days storage) did not appear in the mung bean leaf veins.

As Figure 7, d shows, considerable inhibitory activity appeared in basal xtracts of 'Bartlett' cuttings having *4 buds* each and treated with IBA. This behavior was similar to that shown in Figure 6, c for extracts obtained from disbudded 'Old Home' cuttings treated with IBA. In tests where root formation was measured, both types of such cuttings showed similar poor rooting.

The naturally-occurring, highly-active root-promoting substance which appeared in mid-September in the bases of IBA-treated 'Old Home' cuttings having buds has been studied further (1) and is believed to be an indole-phenol complex arising from the applied auxin combining with a phenolic substance coming from the buds on 'Old Home' cuttings at this time of year. Buds on the difficult-to-root 'Bartlett' cuttings, apparently not only do not produce this rooting factor, but produce strong rooting inhibitors.

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MODERATOR MAIRE: I am Dick Maire, from the Agricultural Extension Service, Los Angeles County, California, and am working with nursery production problems. I am very happy to be here to moderate the second session this morning. We are going to consider the subject of container production of nursery stock and we have a very fine group of speakers. To open, we have Dr. James Kelley. He is from the University of Kentucky where he has been doing work in ornamental research, as well as teaching, for the last ten years or so. He has done quite a bit of work with container production problems and is going to bring us up to date on some of his new ideas in container production of nursery material. The more we can learn about techniques in this field the better. Dr. Kelley:

CONTAINER PRODUCTION OF NURSERY STOCK

JAMES D. KELLEY
Department of Horticulture, Iowa State University
Ames, Iowa

The expanding use of containers for the production of nursery stock has created a need for more information regarding the production of woody plants by this method. Twenty years ago the growing of a plant in a restricted volume of soil was foreign to many nurserymen and many questions were unanswered concerning the cultural practices involved. Today we have a number of answers but still many questions remain to be answered.

Some of the biggest problems have been concerned with fertilization, growing medium, and winter protection. These are some of the items I would like to comment on today and hope that the results of our studies over the past few years may be of value to you in solving some of your production problems associated with growing nursery stock in containers.

Fertilization — Fertilization appears to be one of the least understood factors in growing plants in containers. The purpose of fertilization is to provide an optimum supply of nutrients in order for plants of that particular species to make maximum growth. Many times the desire for maximum growth leads to application of excessive amounts of fertilizer which can be as detrimental to growth as is a lack of fertilization.

Water soluble fertilizers are most commonly used for container-grown stock. However the development of various