UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
CROPS RESEARCH DIVISION
BELTSVILLE, MARYLAND 20705

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Mr. Grote Reber
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Hobart, Tasmania, Australia

Dear Mr. Reber:

Your letter of May 28, addressed to Dr. Howard S. Gentry concerning Dioscorea, has been referred to this office.

For background information on our Dioscorea program, we are enclosing several publications that may be of value to you.

Sincerely yours,

J. R. Haun, Leader
Chemurgic Crop Investigations
New Crops Research Branch

Enclosures
Factors involved in the vegetative propagation of Dioscorea spiculiflora Hemsl. from vines
Preliminary studies of Dioscorea growth in Florida
Relationships of temperature and photoperiod to growth, flowering, senescence, and dormancy of Dioscorea spiculiflora
Biosynthesis of sterols and sapogenins in Dioscorea spiculiflora
Chromosome numbers and behavior in some species of Dioscorea

examined two or three times each week for cracking, visible sprouts, and elongating sprouts. When the sprouts were 1 inch long, the tuber pieces were removed from the flats and planted in the greenhouse.

In general, rotting of tuber pieces was negligible (Experiment I—none; Experiment II—two pieces at 70° F., three at 80° F., three at 90° F.; Experiment III—one in each treatment), but fifteen of eighteen pieces rotted at both 100° and 110° F. (Experiment II). All calculations on sprouting were based on the pieces that survived each treatment. In the 80° F. treatment, Experiment II, six pieces developed cracks or sprouts within 84 days at 80° F.; the remaining nine pieces were then planted at 90° F. along with all pieces (none of which were sprouted) formerly at 70°, 100°, and 110° F.

Responses shown in figure 5 are expressed in terms of 50% of the tuber segments approaching a given visible stage of development; this point is derived from a curve (such as shown in fig. 7) made up of data from all tuber pieces involved in the treatment and is an expression similar to the familiar LD_{50}.

PHOTOPERIOD TEST.—Two adjacent greenhouse sections containing benches with *D. spiculiflora* plants (seedlings) growing in peat were selected for the photoperiod treatments. An interrupted-night cycle (2-hour incandescent illumination from 12 P.M. to 2 A.M. EST) was begun in one section on October 1, 1959. Illumination at night was supplied by six 100-watt lamps suspended above the 50-foot benches. Black building paper was placed on the glass wall between the two sections to prevent night illumination of the plants in the control section.

Portions of vines in both groups were removed for propagation, and these plants were used for comparisons in this study. All but the lowest four to eleven leaves on each plant were removed. In the section receiving night illumination, 104 vines were cut back and used for propagation during the period October 14–24, 1959. Twenty-five of the 104 vines had already developed one or more small flower clusters by October 1, 1959. Fifteen of the twenty-five vines were cut back far enough so that no flower buds were apparent; a few flower buds were visible on the other ten vines. In the section exposed only to normal (short) days, forty-two vines were cut back during the period September 16–October 22, 1959. Only one of these vines had developed small flower clusters at this time.

All vines, after being cut back, were from 8 to 20 feet in length and were supported on wires between the lamps. The vines that produced additional growth developed axillary shoots up to 50 feet long during the course of the experiment. Thus one plant could be exposed to light from two to twelve lamps at various distances and intensities.

Since growth of all plants slowed considerably during late December and early January, a 20-20-20 water-soluble fertilizer (Nutrileaf) was sprayed on the foliage at the rate of 1 lb/20 gal on January 13, 1960. Then an application of 5-10-5 fertilizer was made to the peat medium at the rate of 1 lb/100 sq ft on January 20, 1960.

Results and discussion

TEMPERATURE TREATMENTS WITH TUBER SEGMENTS

LENGTH OF DORMANT PERIOD.—Typical development of sprouts on tuber pieces of *Dioscorea spiculiflora* planted at constant 90° F. is represented in treatment 1, Experiment I (fig. 5) and in figure 7. These pieces were from stock tubers with completed dormant period. Tuber pieces in treatment 1, Experiment II, (fig. 5) were from actively growing plants and had to complete the dormant period before sprouting. From comparisons of speeds of sprouting on tuber pieces in the groups with and without the dormant period, the approximate length of the dormant period appeared to be 2–3 months. This was estimated by subtracting the number of days (31) for development of cracks in treatment 1, Experiment I, from that (58) in treatment 1, Experiment II, or the number of days (54) for development of 1-inch sprouts in treatment 1, Experiment I from that (105) in treatment 1, Experiment II, then adding the preliminary 31-day 70°–75° F. holding period in treatment 1, Experiment II to each difference; the resulting totals were 58 and 82 days.

TEMPERATURE OF DORMANT PERIOD.—No tuber pieces sprouted during the 84-day exposures at 110° and 100° F., very few (discussed later) at 80° F., and none during the 97-day exposure at 70° F. (treatments 2–5, Experiment II, fig. 5). At 90° F. (treatment 1, Experiment II), however, 50% of the tuber pieces had developed cracks within 58 days and visible sprouts within 79 days. Therefore, all pieces in the 110°, 100°, 80°, and 70° F. groups were then placed at 90° F. (final treatments 2–5, Experiment II), where they sprouted rapidly. This response indicated that the tubers could complete the dormant period at any of the temperatures used (70°–110° F.). It also indicated that 90° F. was near the optimum for sprouting and was within a rather limited temperature range that would permit it.

Sprouting at 90° F. was progressively more rapid the higher the temperature during the previous exposure periods (treatments 2–5, Experiment II, fig. 5). This response might be attributed to possible changes in the tuber pieces in preparation for sprouting (without actual cracking or sprouting taking place because of unfavorable temperature). If this hypothesis were extended, then 110° F. was most conducive to these preparatory changes, and 100°,

Typical tuberous-root system of Dioscorea composita, PI 202324 after three years in planting.

from P. I. 200118 doubled in weight while those of P. I. 202324 and P. I. 209279 exhibited a sixfold average weight increase over the first year. During the third year, the greatest weight increase of tuberous roots was obtained from P. I. 202324; followed in descending order by P. I. 209279 and P. I. 200118.

Plants in the supported row gave consistently higher average weights of tuberous roots than plants in unsupported rows, and in many instances these weight differences amounted to more than a third of the reported weight of plants in unsupported rows.

Progressively higher percentages of sapogenin content were obtained each year up to the second-year harvest for P. I. 209279 and up to the third-year harvest for P. I. 200118 and P. I. 202324.

As a whole, the seedling planting of P. I. 209279 did not produce as much tuberous-root growth, as the vegetatively propagated introduction of P. I. 202324 and it did not have as high a percentage of sapogenin.

Insect and disease problems did not develop enough to warrant control measures. However, some plants showed minor injury from aphids and root-knot nematodes. It is believed that root-knot nematodes might present a serious problem if planting was carried out in heavily infested soil.

Summary

Following 3 years of growth in trial field plantings, it was found that substantially greater weight increases of tuberous roots occurred in the second and third years of growth than in the first year. In the third, or final, harvest the greatest weight gain was obtained from *Dioscorea composita,* P. I. 202324. Weight gains of *Dioscorea* sp., P. I. 209279 and *D. floribunda,* P. I. 200118 followed in descending order.

Sapogenin content of P. I. 200118 and P. I. 202324, increased each year up to the third-year harvest and that of P. I. 209279 up to the second-year harvest.

The data suggest that *D. composita,* P. I. 202324 is better adapted to growth under the environmental conditions of this trial. Further, a greater yield of sapogenin can be expected if vine supports are provided than if no supports are provided.

LITERATURE CITED

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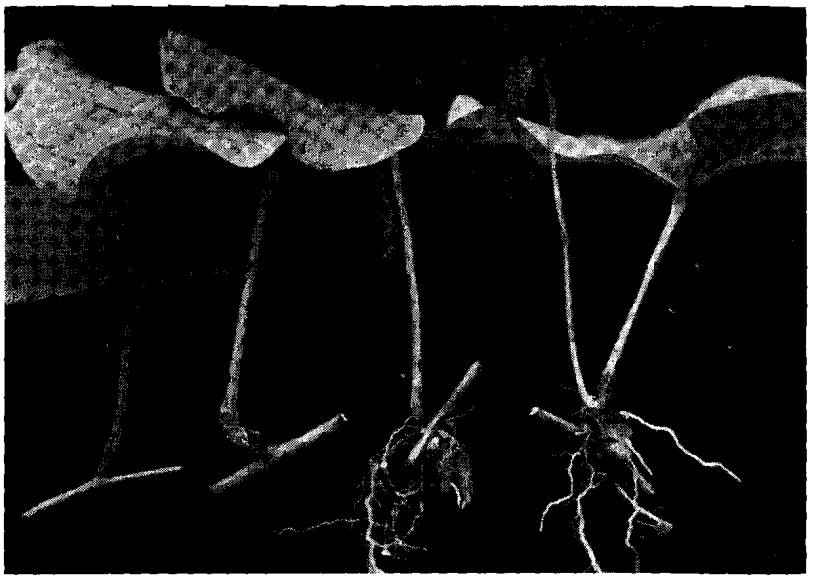

Fig. 3. Development of new miniature plants from axillary buds of vines of *D. spiculiflora* (left to right): (a) one-noded segment as severed from vine; (b) swelling and parting of folds at base of petiole; (c) tuberous and fibrous roots developed from the axillary bud; (d) new tuberous root with fibrous roots and a shoot.

The stock plants were grown on greenhouse benches containing European peat supplemented occasionally with 4–8–12 fertilizer. From September through March the plants were grown at 60° F minimum night temperature and were exposed to 2 hours of light each night, beginning at midnight. The vines or vine segments were severed from the stock plants and set in quartz sand in a mist propagation greenhouse. The misting cycle was 1 minute of fine mist followed in 2 minutes by 8 seconds of misting during every 6 minutes of the day, with no misting at night. The stems of all cuttings were laid horizontally approximately 1 inch beneath the surface of the sand with the leaves exposed to the mist. Heating cables 3 inches below the surface of the medium were thermostatically controlled at 75° F. Minimum air temperature was 70° F. From September through March, all cuttings propagated in mist received 2 hours of incandescent illumination beginning at midnight. Occasional minor infections of *Botrytis* on the leaves of the cuttings in mist were kept under control by removal of infected portions when detected. Also any necrotic areas on leaves and stems of cuttings prior to placing in mist were removed to prevent further necrosis in the propagation bench.

Propagation Tests

Types of Cuttings. Descriptions of the types of cuttings used are listed in Table 1.

In two lines of *D. floribunda*, the 2n chromosome number was 54, and up to 9 quadrivalents were seen (Fig. 1). In this case, meiosis was characterized by the presence of various abnormalities (Table 3), including univalents, bridges, fragments, and delayed separation of bivalents. It was not possible to determine whether the bridges were due simply to sticky, lagging chromosomes or breakage was involved. The presence of acentic fragments suggests that the latter process sometimes occurred. Subsequent stages of meiosis in this line were normal. Cytokinesis occurred after second metaphase. Univalents and fragments probably were usually included in macronuclei, as micronuclei were seldom observed.

Fig. 1. Mitotic (above) and meiotic (below) chromosomes in *Dioscorea floribunda*. Left, a 36-chromosome line; right, a 54-chromosome line (×1000, from photomicrographs and camera lucida drawings.)

Mitosis. Obtaining chromosome counts from root tips was much easier and more reliable than obtaining meiotic counts. Nevertheless, at times a preponderance of cells with well-developed prochromosomes of the vesicular type made the actual search confusing and tedious. The mitotic counts of sapogenin-bearing species were in agreement with the meiotic counts, but many more collections and species were counted (Table 2). The chromosome numbers of all Old-World species counted are multiples of 10. These counts are in agreement with those of previous investigators. The chromosome numbers of the New-World species are multiples of 9. The numbers of these species have not been previously reported, with the exception of an approximate count of *D. spiculiflora*. The chromosomes were small and dotlike (0.5 to 2.0 microns). No consistent heterotypic elements were found.

Table 3. Meiotic abnormalities in hexaploid *Dioscorea floribunda*

Number of cell	Stage of meiosis	Univalents	Laggards	Bridges	Fragments
1	Telophase	2	0	2	2
2	Telophase	3	0	2	0
3	Late anaphase	2	0	5	3
4	Late anaphase	0	1	3	2
5	Anaphase	0	1	5	2
6	Anaphase	1	0	3	0
7	Late anaphase	0	0	2	3
8	Early anaphase	2	3	0	0
9	Early anaphase	1	2	0	0
10	Early anaphase	0	4	0	0

The data on chromosome numbers presented here, together with the scanty previous observations, suggest that the progenitors of the New-World

of each absorbed by the plant. This actually represents a greater incorporation of mevalonic acid, since only half of the radioactivity of the racemate is available for biosynthesis. Moreover, the incorporation of mevalonic acid into the crude sapogenin fractions represented 0.2% of the radioactivity absorbed, whereas the incorporation of acetate was only 0.09%. Thus, the failure of mevalonic acid to be a more efficient steroid precursor than acetate in homogenates must be ascribed to other factors (14).

Comparison of the administration of mevalonic acid via the roots, leaves, and stems showed that the latter route is by far the most efficient. These results suggest a more efficient translocation of the precursors to the major sites of steroid biosynthesis via the stem. Incorporation of mevalonic acid into the lipids of the shoot system was higher than in the tubers, regardless of the route of administration, but most effective after administration via the stem. The biosynthetic activity of the shoot system is not only higher than but qualitatively different from that of the tubers. The most striking difference is the extremely high radioactivity (almost half of the total) in Fraction 1 of the shoot lipids (Fig. 2), while in the tuber the radioactivity of this fraction is relatively low (9% of the total).

As Table I shows, the total radioactivity of the lipid extracts was about five times as great in the shoot system as in the tubers. Most of the activity in the tubers was found in Fractions E and F, the neutral extracts of the hydrolyzate, whereas in the shoot system it was found to be almost equally divided between Fractions D and E. Fraction D is largely composed of highly nonpolar material, probably unsaturated hydrocarbons such as carotenoids.

The fact that the steroids were found only in the extracts of the hydrolyzates (Fraction E) suggests that in the plant they exist wholly in the form of conjugates, such as glycosides. It is interesting to note that in both the tuber and shoot system the extract with the highest specific activity was Fraction G, which represents basic material from the hydrolyzate. The true specific activity of these fractions was even higher, because they were somewhat volatile and the radioactivity decreased continuously as they were counted.

Table III shows that while the amount of all steroids in the shoot system is much lower than in the tubers, the specific radioactivity is generally much higher. This indicates that the shoot system is the major site of biosynthesis of steroids and that the synthetate is rather rapidly translocated to the tuber.

The relationship between the radioactivities of β-sitosterol and stigmasterol (Table III) is rather surprising. Both the specific activity and the total activity of the former are much higher in both systems of the plant.[11] This would indicate that, if there is a sequential relationship in the biosynthesis of the two sterols, stigmasterol is formed by dehydrogenation of β-sitosterol in the side chain. This is contrary to the pattern observed in the biosynthesis of animal sterols, where unsaturated compounds are precursors of the more saturated sterols.

Although the radioactivity of cholesterol in the shoot system could not be determined accurately, the figures given in Table III are probably not greatly above the actual radioactivity. The extremely high specific activity of this sterol, together with the small amount present, suggests that it may function as a precursor, perhaps of the sapogenins (1).

A comparison of the specific activities of the sapogenins isomeric at C-25 (Table III) shows that in all cases the 25α isomer has a higher specific activity than the 25β isomer, indicating that they are either formed from a common precursor or that the former precedes the latter.

The dehydration of sapogenins to $\Delta^{3,5}$-dienes under conditions of acidic hydrolysis (11) presents a problem in a quantitative study such as this one. We were able to diminish the artifact formation by the use of a layer of benzene into which the sapogenins were extracted as the

[11] This relationship between the radioactivities of β-sitosterol and stigmasterol has also been observed in *Solanum tuberosum* (D. F. Johnson, unpublished) and *Xanthium pennsylvanicum* (E. Heftmann, unpublished) plants.