of the flavor of cooked beef. Mussinan et al. (1976) suggested that 2,4,5-trimethyl-3-oxazoline can form in heated meat systems by the thermal interaction and rearrangement of the compounds, ammonia, acetaldehyde, and acetoin. Alternatively, a study of the Strecker degradation shows that 2,4,5-trimethyl-3-oxazoline might be formed from alanine and 2,3-butanedione (Rizzi, 1969; Ohloff and Flament, 1979). Among the three oxazolines identified in the volatiles of roasted peanuts, 2-methyl-3-oxazoline has never been reported in food flavor. 2,4,5-Trimethyl-3-oxazoline has woody and green aromas. 2-Methyl-3-oxazoline and 2,4-dimethyl-3-oxazoline were described as nutty and sweet.

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Preliminary Results of in Vitro Propagation of Guayule

The propagation of whole guayule plants from tissue culture is desirable but has not yet been achieved. For facilitation of this effort, conditions which optimized tissue culture growth using MS media were investigated. In addition, preliminary results of the use of a chlorophenoxy derivative of triethylamine as a possible growth hormone are presented. The derivative was shown to alternately produce shoot or callus formation, exclusive of each other, as a function of concentration.

Guayule, *Parthenium argentatum* Gray, was first established in tissue culture by Bonner (Bonner and Arreguin, 1950) as a means to study the effects of various chemicals and extracts on rubber production. The propagation of whole guayule plants from tissue culture, however, has not been accomplished, and our efforts have been directed toward this end as a means of increasing rubber production through genetic manipulation. Initial experiments designed to define the optimal growth conditions for guayule revealed significantly different responses to alterations in media and hormone levels. In an attempt to stimulate rubber production in guayule, Yokoyama et al. (1977) sprayed juvenile plants with 2-(3,4-dichlorophenoxy)triethylamine (TEA derivative). This treatment resulted in increased isoprenoid levels in the plant tissue. Since its effect was pronounced on juvenile guayule plants, it appeared worthwhile to ascertain its effect on in vitro cultures. In the process of examining these possible effects, interesting hormonal properties were discovered. A suppression of callus formation occurs at a 10 mg/L concen-

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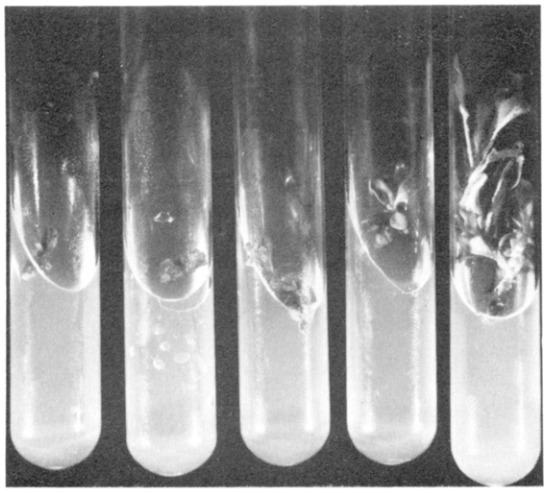

Figure 1. Callus and shoot formation. Explants were obtained from the tissue described in the text. Stem tissue was cut into 2–5 cm long segments, stripped of leaves, and washed in detergent with gentle brushing. These segments were then sterilized for 15 min in 5% commercial bleach, cut into two to three pieces ~0.5–2 cm long, and used to inoculate slants containing different media. Tubes 1–3 were formulated as the B medium in Table I, and tubes 4 and 5 were formulated as the media E and F in Table I. Tube 1 is an initial inoculation, and tube 2 is a callus at inoculation plus 7–10 days. Tubes 3–5 are shown as they were found 30–32 days after inoculation. Tube 3 has both callus and shoot, but tubes 4 and 5 have only shoots.

tration of TEA derivative with a resultant increase of shoot formation. Lower and higher concentrations of TEA derivative promote callus formation.

Explants for tissue culture work were obtained from juvenile guayule plants that ranged 15–50 cm in height and were 3–9 months old. Shoot tissue was obtained from several sources including shoot tips, sections from young shoots, and more mature stems.

The basal media used for all experiments included those of Murashige and Skoog and Schenk and Hildebrandt as described by Gamborg (Gamborg et al., 1976).

Callus formation in stem segments of both nodal and internodal regions usually showed cell division in the pith and cambium regions primarily above but occasionally below the agar line. Formation of callus occurred after 7–10 days. Adventitious shoots developed in stem tissue at internodal and nodal points and at the end of segments above the agar line. Figure 1 depicts callus and shoot formation. The addition of the TEA derivative provided a luxuriant growth of shoots (Figure 1, tubes 4 and 5). Some shots were maintained up to 4 months.

Hormone levels, auxin/cytokinin ratios, pH, sucrose levels, and TEA derivative concentrations used and the resulting formation of guayule callus and shoot are shown in Table I. Inspection reveals a number of differences in callus and shoot formation as a function of the variables described. The results of an analysis of variance and the *F* test show a greater than 1% confidence level for callus formation and a greater than 5% level for shoot formation.

These preliminary data indicate that callus/shoot formation in guayule can be controlled by altering IAA and kinetin levels and ratios (Table I, B–D), as well as by the use of other media formulations (Table I, G). The increased shoot formation (Table I, E) and very low callus formation (Table I, columns E and F) posed an interesting question as to the effect of the TEA derivative on guayule tissue.

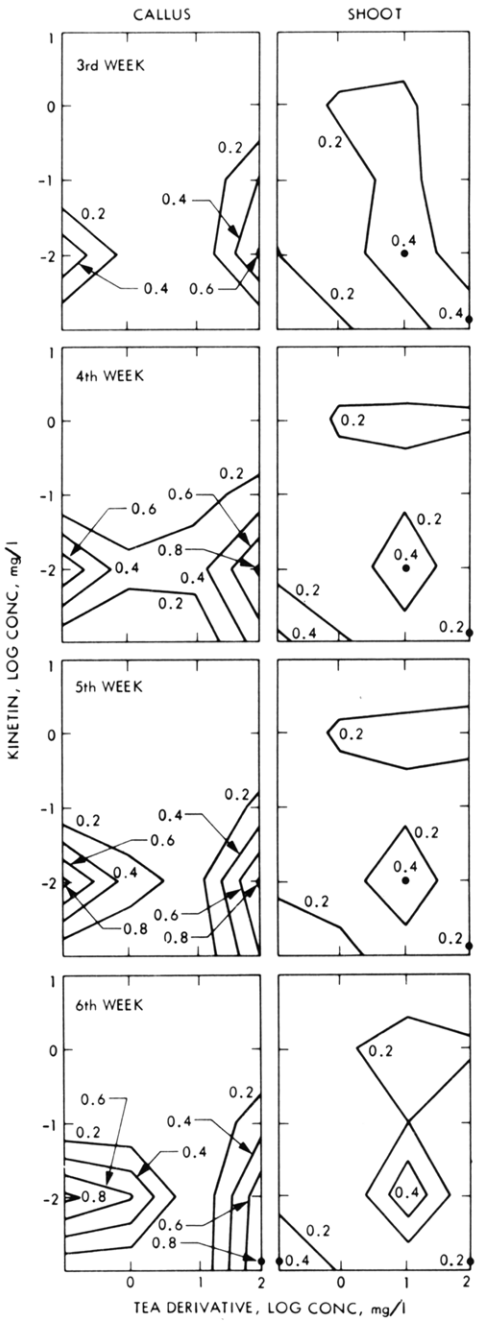

Figure 2. Patterns of callus and shoot formation in guayule tissue. This figure is a three-dimensional representation of the observations made in the matrix. The matrix includes 20 separate observations that were conducted simultaneously. Each of the 20 observations utilized a different kinetin and TEA derivative level with 10–15 separate slants containing tissue per observation. The basal media was an MS media (see E and F in Table I) and growth conditions were as described in Table I. The mean average of the fraction of slants containing callus or shoots found for 3 successive weeks of observation commencing 21 days after inoculation was measured. Thus, the observations were conducted at 3, 4, 5, and 6 weeks after inoculation. Each week of observation of the matrix experiment was plotted on a contour plot. The contour lines connect the observations which have identical levels of callus or shoot formation. The numerical value ascribed to each contour line indicates the fraction of callus or shoots in the observation. The uppermost set of graphs represents the observation at 3 weeks after inoculation, and each succeeding set of plots represents observations at 1 week (±2 days) after this initial observation. Infected slants are removed from consideration as they occur, and thus the low level of callus at the fourth week in the 10 mg/L TEA range disappears in succeeding weeks. The significant factor is that at the other derivative concentrations, new callus formation increases while at this specific level no new callus formation occurs.

Table I. Effects of Different Media on Callus and Shoot Formation[a]

	A	B	C	D	E	F	G
callus	0.80 ± 0.23	0.84 ± 0.15	0.94 ± 0.10	0.02 ± 0.04	0.05 ± 0.10	0.00 ± 0.00	0.90 ± 0.22
shoot	0.09 ± 0.11	0.09 ± 0.14	0.18 ± 0.17	0.51 ± 0.22	0.64 ± 0.42	0.12 ± 0.13	0.05 ± 0.11
root							
IAA, mg/L	4.0	4.0	1.0				
kinetin, mg/L	2.56	2.56	0.04	0.1	0.1	0.1	
2,4-D, mg/L							0.5
pCPA, mg/L							2.0
TEA derivative, mg/L					10	1	
basal media	MS	MS	MS	MS	MS	MS	SH
pH	5.7	4.9	5.7	5.7	5.7	5.7	5.7
sucrose, g	30	15	30	30	30	30	30

[a] Each value represents the mean average of at least 3 separate observations with each observation comprised of a group of 2-40 slants containing stem tissue. The standard deviation is included. The group of slants were randomly placed in a growth area illuminated on a light/dark ratio of 16:8. The temperature and humidity varied during this cycle between 29 °C and 31% humidity during light cycles and 25 °C and 35% humidity in the dark cycles as measured by a Foxboro temperature and relative humidity meter. The slants were scored after a minimum of 14-days incubation and the fraction of tubes containing callus and/or shoots was recorded.

For quantification of the effect of the TEA derivative on callus formation and organogenesis, a matrix experiment was conducted to test the action and interaction of kinetin and TEA over a broad range of concentrations. The F test revealed a confidence level of 5% for the data produced in the entire matrix. In an effort to identify the location of differences with this limited data, a contour plot of the data was made. Figure 2 shows the results of this plotting.

The most significant result of this plotting is shown by comparing callus and shoot formation as a function of TEA derivative concentration. Shoot formation was greatest at 10 mg/L TEA derivative and is somewhat independent of the kinetin levels used. At this level callus formation was strongly inhibited. This derivative compound thus produces a concentration-dependent inhibition on callus formation that concomitantly produces an increase in shoot formation. This result provides possible evidence for an unique hormonal action.

Further experiments to better define the effect of the TEA derivative on guayule tissue in vitro are needed. No further experiments have yet been conducted to test the effect of TEA derivative in synergism with any other growth hormones.

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The Presence of Damascenone in Cultivars of *Vitis vinifera* (Linneaus), *rotundifolia* (Michaux), and *labruscana* (Baily)

Damascenone, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one, was identified in several cultivars of three species of grapes (*Vitis*). Because damascenone has a pleasant floral odor and a very low threshold in water, its concentration in extracts of juice and wine of several cultivars of *Vitis* was determined. The highest concentration was found in Concord, a cultivar of *Vitis labruscana*.

The odor of *Vitis* species *vinifera*, *labruscana*, and *rotundifolia* is very different and easily recognizable. Although hundreds of odorous compounds have been found in grapes (Schreier, 1979), only methyl anthranilate seems unique to one specie, *labruscana*. However, methyl anthranilate has been found in only a very few of the cultivars of *labruscana* grapes (Sale and Wilson, 1926; Nelson et al., 1977a). Damascenone, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one, was identified in wines of a *Vitis vinifera* cultivar, Riesling, (Schreier and Drawert, 1974) and quantified in the grapes and wines of two cultivars of *labruscana* grapes (Masuda and Nishimura, 1980).

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