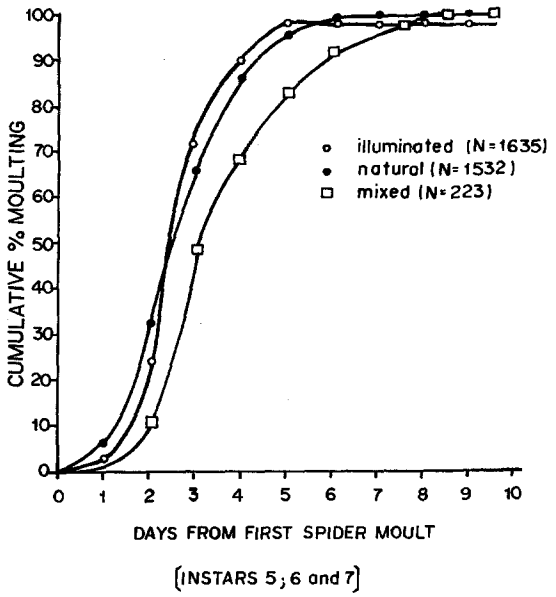

Figure 3. Molting rates of natural, back-illuminated, and combined colonies. See figure 2 for more details.

to reduce the risk of cannibalism, as generally larger individuals prey upon smaller ones. In fact, cannibalism is never observed in *E. bistriata* in the field[3].

For *E. bistriata,* synchronizing the molting cycle could also have a further advantage, that of permitting cooperative prey capture[10]. *E. bistriata,* like all araneids, constructs individual orb-webs nightly for prey capture. However, extraordinarily large prey can be ensnared in individual orb-webs, and captured through the cooperation of neighboring spiders which enter the individual orb-web to subdue the prey[10]. Although cooperative prey capture is common in species which construct a collective web[11], this behavior among orb-web spinning spiders is unique to *E. bistriata*[10], and is probably coordinated by vibrational stimuli perceived by neighboring spiders. These vibrational stimuli are probably interpreted allometrically, depending upon spider size. The possibility of recognizing spiders from neighboring webs could facilitate communal prey capture, while, at the same time, minimizing potentially fatal aggressive confrontations in the individual orb-webs, which are defended unless an extremely large prey is ensnared[3].

We suggest that molting is synchronized by chemical communication, probably transmitted during the diurnal roosting period, when all spiders are in close physical contact. Whether this chemical cue is a primer pheromone, or the liberation of molting hormones perceived on a newly molted individual, its effect is quite clear-cut, leading to a high degree of colony molting synchrony.

Acknowledgment. We thank Elena Fowler for her help during these studies. Partial funding for these studies were provided by UNESP. We thank M. Petrere, C. Sandoval and L. C. Forti for their comments on the manuscript. Nozor Pinto kindly penned the figures.

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0014-4754/88/080720-03$1.50 + 0.20/0

Adventitious juice vesicle initiation in lemon (*Citrus limon* L.), mandarin (*Citrus reticulata* Blanco), sour orange (*Citrus aurantium* L.), and sweet orange (*Citrus sinensis* (L.) Osb.) fruit explants

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Received 19 January 1988; accepted 4 May 1988

Summary. Adventitious juice vesicles have been obtained from lemon, mandarin and navel and sour orange juice vesicle explants cultured for prolonged periods on a nutrient medium containing 3.0% sucrose in vitro.
Key words. Aurantioideae; callus; maturation; morphogenesis; proliferation; Rutaceae; tissue.

The citrus fruit is unique among the angiosperms[1]. The fruit is a berry (hesperidium) consisting of 6–20 united carpels. These carpels are oriented vertically with their margins curved adaxially to join the floral axis thus forming locules. Exterior to the locules is the pericarp which is subdivided into three regions: the exocarp (flavedo or exterior peel), masocarp (albedo or interior peel), and the endocarp (locule membrane). Juice vesicles arise from primordial bumps on the surface of the endocarp. Through cell division they differentiate into elliptical shaped juice vesicles consisting of a distinct stalk and a single terminal body with tapered ends. Citrus vesicles are characterized as being uniform solitary stalked structures which accumulate juice[1]. Since the juice vesicles are the edible portion of the citrus fruit they are of great economic value.

Other investigators have attempted to culture either individual juice vesicles or fruit explants containing juice vesicles on nutrient medium containing high concentrations of carbohydrates and growth regulators[2–6]. Past studies were short-term (typically less than 30 days) and invariably resulted in the deterioration of the original vesicle tissue into a callus mass. Described herein, is a long-term fruit bioassay system to analyze the effects of chemical and physical environments on citrus fruit tissue growth and metabolism in vitro. In the

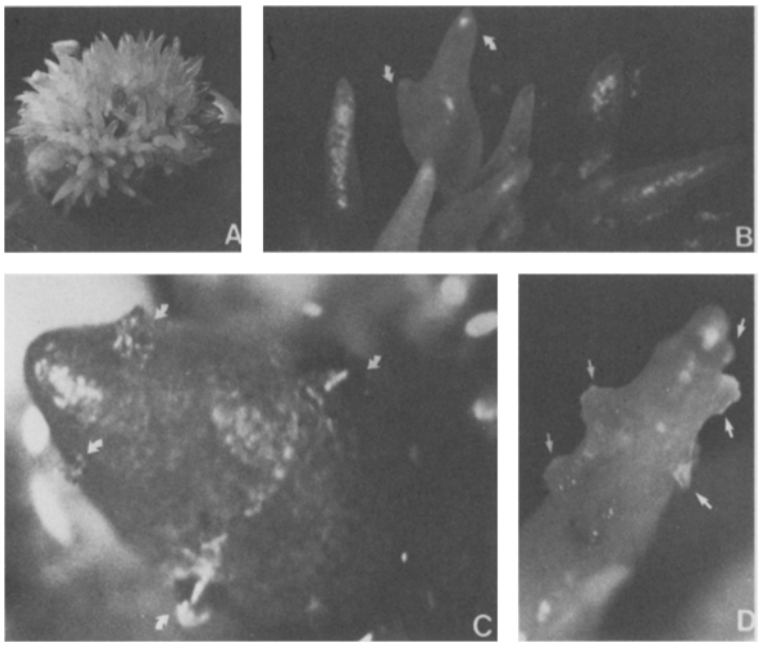

Figure 1. Examples of citrus juice vesicle growth forms. *A* A lemon fruit half after 60 days in culture. Note that vesicles are still intact and growing out of fruit locule into the atmosphere. *B* A known lemon tip primordium bifurcates to form two vesicles each with their own primordium tip but attached to the same stalk. *C* A navel orange juice vesicle gives rise to multiple adventitious juice vesicle primordia. *D* A mandarin juice vesicle gives rise to multiple adventitious juice vesicle primordia. White arrows identify adventitious vesicles or vesicle primordia.

course of studying prolonged vesicle growth in vitro we discovered adventitious vesicle structures arising from preformed vesicles.

Materials and methods. Fruit, 10–15 mm in diameter, of *C. aurantium* L. (sour orange), *C. limon* (L.) Burm. f. cv. 'Eureka' (lemon), *C. sinensis* (L.) Osb. (navel orange) and *C. reticulata* Blanco (mandarin) were surface sterilized with 2.63% NaCl for 30 min, halved and planted in nutrient medium. The fruit flavedo was embedded in agar nutrient medium and the endocarp exposed to the atmosphere (fig. 1 A). The nutrient medium contained the following in mg l^{-1}: KNO_3, 100; H_3BO_3, 2.5; $CaCl_2 \cdot 2\,H_2O$, 150; $CuSO_4 \cdot 5\,H_2O$, 1; $FeSO_4 \cdot 7\,H_2O$, 3; $MgCl_2 \cdot 6\,H_2O$, 97.8; $MgSO_4 \cdot 7\,H_2O$, 100; $MnSO_4 \cdot H_2O$, 2.5; KI, 0.5; KH_2PO_4, 5; $Na_2MoO_4 \cdot 2\,H_2O$, 0.25; $ZnSO_4 \cdot 7\,H_2O$, 2; thiamine · HCl, 0.5; i-inositol, 100; sucrose, 30,000; and agar, 8,000. Cultures were incubated at a constant 26 ± 1 °C under a 16-h daily exposure to 2.2 Wm^{-2} cool white fluorescent lamps. After 60 days in culture, explants were removed and placed in 3% glutaraldehyde[7] and embedded in Polaron glycol methacrylate polymer plastic[8] for histological examination. Specimens were sectioned at 3 µm and stained with 0.1% toluidine blue.

Results and discussion. Initially, vesicles within the cultured fruit half were conical in shape and green in color. These vesicles began to enlarge and expand out of the locule cavity within a few days after planting on nutrient medium. Within a week, vesicles were observed to form a bristle pattern emanating from the fruit. As the vesicles matured in culture they developed a distinctive elliptical form and eventually lost their green coloration to become opaque (e.g. lemon) or yellow-orange (e.g. mandarin and orange) (fig. 1 A). Vesicles have been grown in culture as intact tissue appendages for as long as 8 months without loss of original integrity.

Mass callus formation from cultured vesicles was avoided in our cultures by carefully minimizing surgical injury to the vesicles during the excision procedure. Injury to the vesicles resulted in a wound-healing response which preceded the formation of callus[3,4]. In citrus, this callusing response is irreversible and can result in the proliferation of a callus which submerges non-callusing vesicles and thus significantly alters normal vesicle development from cultures.

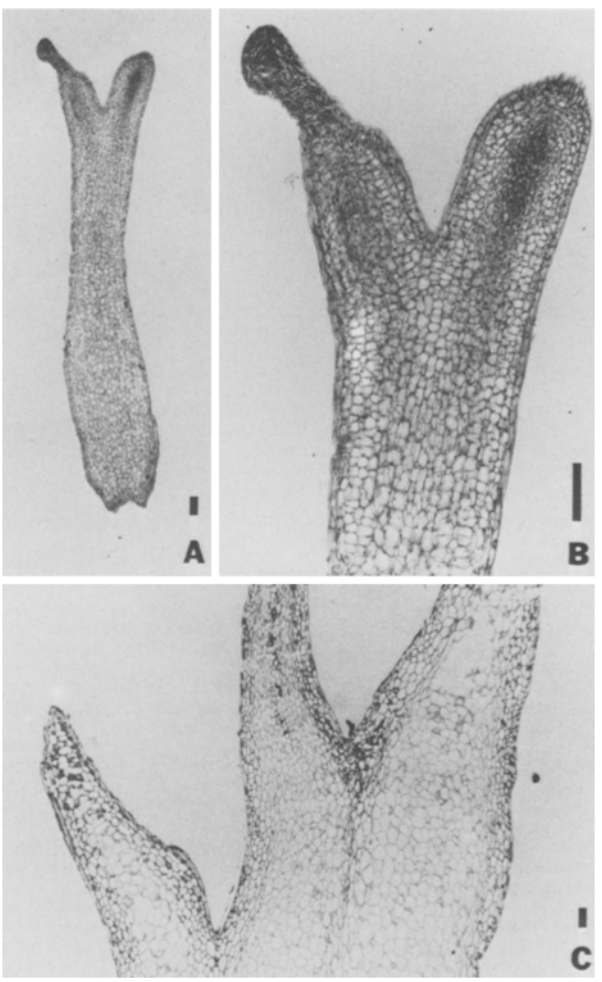

Figure 2. Histological sections of juice vesicles with adventitious primordia. *A* Cross section of a sour orange vesicle with a branched apical tip. ×25. *B* Enlargement of branched tip of sour orange vesicle. ×75. *C* Lemon juice vesicle with three distinct tips emerging from apical tip. ×25. Scale bars = 100 µm.

Juice vesicles in cultured immature citrus fruit halves sometimes form adventitious juice vesicle structures when incubated on our nutrient medium containing 3% sucrose (fig. 1 B, C and D). Adventitious vesicles were produced from either preformed appendages emerging from the epidermal surface of existing vesicles or through the bifurcation of the vesicle tip primordium (figs 1 and 2). Histological sections reveal that these adventitious vesicles have the distinctive outline and internal cellular connections as the original apex tip of the vesicle (fig. 2). Adventitious vesicles could sometimes be induced to develop as normal vesicles (fig. 1 B). These adventitious vesicles were infrequent, only occurring in less than 1% of all vesicles cultured. However, one or more adventitious vesicles were found to occur in about 5% of the cultured fruit halves. Fruits of various chronological ages obtained from the tree for these same species were found to be devoid of any adventitious vesicles. Adventitious vesicle induction appears to be an in vitro only response of the cultured fruit to the sterile environment. Adventitious vesicles were not found to be a continuous source of vesicles; only a few additional vesicles may arise from them. As vesicles age in culture their ability to produce adventitious vesicle primordia decreases proportionally. However, the fact that it can occur at all, leads one to suspect that through further investigation a proliferation scheme could be developed.

This work demonstrates that new vesicles arise from preexisting ones maintained in culture.

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