day access; and two, 12-day access. The surviving nine leafhoppers and five noninoculative leafhoppers were examined with the SEM (*15*). The pump diaphragm with muscles attached (Fig. 1B) was separated from the cibarium so that the food meatus to the stylets was dorsally visible (Fig. 1C). The entire cibarium was examined, and rod-shaped bacteria were found only in those areas in which they had previously been detected with light microscopy. Seven of the nine infective leafhoppers showed rod-shaped bacteria in the medial groove at the base of the apodeme of the cibarial pump diaphragm (Fig. 1B) and five of the seven showed bacteria in the "food meatus" of the cibarium (Fig. 1, D and E). Bacteria were not found in one leafhopper from each of the 1- and 4-hour postacquisition access periods, nor in the five noninfective leafhoppers (Fig. 1F). Significantly, the bacteria were not loosely scattered, but formed distinct colonies (Fig. 1, D and E). In general, the colonies were larger and denser 4 and 12 days after acquisition feeding. The bacteria attached themselves on their narrow end on the smooth interior surface of the cibarium; this attachment appears to prevent their being dislodged despite the flow of ingested fluid estimated at an average velocity of 8 cm/sec (*16*). The matrix surrounding the bacterial aggregates, which was quite evident in paraffin sections, may be a gelatinous material that leaves only a thin residual film in the freeze-drying preparation for SEM (Fig. 1E). The matrix material surrounding the PD bacterium may aid their attachment and protection. Notably, we found only one other bacterium—a coccoid *Lactobacillus*-like organism, possibly *L. hordniae* (*10, 17*)—was found attached to the foregut. The cibarium of xylem feeders could be expected to be a highly selective microenvironment because of its low nutrient concentration and high flow velocities. Thus, bacteria which colonize such habitats should be able to securely attach to these surfaces.

The location and apparent multiplication of the PD bacteria in the foregut suggests that the bacterial pathogen is propagative, but noncirculative in its insect vector. The force of the pumping action and liquid flow probably dislodge some of the bacteria, which are then egested through the food canal to infect the plant. This mode of transmission would adequately explain the broad but well-defined spectrum of PD vectors (*1, 2*), the loss of inoculativity by vectors following molting (*8*), the lack of a required latent period in the vector (*8*), and the lack of bacterial transmission by *G. atropunctata* after injections of bacterial suspensions into the body cavity (*3, 18*). Multiplication of the PD bacterium in the foregut would account for the indefinite persistence of inoculativity in vectors that do not molt (that is, adults) (*2, 7*). The wide plant host range (*4*) and the multiplication of the PD bacterium in the distinctly extracellular environment of the leafhopper foregut casts further doubt on the affinity of the PD bacterium to rickettsiae, as suggested on the basis of the bacterium's fastidious habitat and morphological features (*14*).

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15. The head, with thorax attached, was severed from leafhoppers and placed immediately in 4 percent glutaraldehyde-phosphate buffer (*p*H 7.4) for 24 hours, dehydrated in an ethanol series, and held in amyl acetate for 24 hours prior to drying in liquid CO_2 transition fluid. The dried specimens were dissected to remove the cibarium and attached clypeal muscle. The exposed ventral and dorsal cibarium were mounted on stubs, sputter-coated with gold, and viewed in an Etec Autoscan SEM.
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Prevention of Monocarpic Senescence in Soybeans with Auxin and Cytokinin: An Antidote for Self-Destruction

Abstract. *Foliar applications of α-naphthaleneacetic acid, together with 6-benzyladenine, prevent the seed-induced degeneration (monocarpic senescence) in soybeans. In addition to halting leaf yellowing and shedding, this treatment prevents the loss of starch and nitrogen that occurs during senescence of these leaves. Although nitrogen and starch are normally redistributed to support pod growth, pod development is not impaired by this treatment, apparently because photosynthesis and nitrogen assimilation continue.*

During the final phases of fruit maturation, soybean plants rapidly degenerate (monocarpic senescence) and then die. Most prominent among these changes is the yellowing and the shedding of the leaves. Since this abrupt degeneration of the whole plant is controlled by internal factors, it is a developmental self-destruct program. Because defloration, depodding (*1*), and even deseeding (*2*) can prevent monocarpic senescence in soybeans, the seeds are the primary control centers. The primary targets appear to be the leaves, whose death causes the demise of the rest of the plant (*3*).

Many factors limit vegetative development and seed production in soybeans; however, the fact that the basic life-support organs (for example, the leaves) and processes (such as photosynthesis), as well as other processes (such as nitrogen fixation), required to support seed growth degenerate at a time when they appear to be needed most to support reproductive development (*4, 5*) suggests that it could be of both practical and theoretical interest to prevent this decline through applications of a plant growth

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regulator. We now report the prevention of whole organism senescence, specifically monocarpic senescence, through chemical treatments.

Although the surgical procedures mentioned above and others (*6*) have retarded or even stopped senescence of some monocarpic plants (*1-7*), the hormones (such as cytokinins) that are very effective in delaying senescence of detached leaves often have not been effective on the leaves that are still attached (*5*). It has been reported that cytokinin treatments may delay senescence of attached leaves from several species including soybean (*8, 9*). A synthetic auxin, α-naphthaleneacetic acid (NAA), may also inhibit monocarpic senescence in soybean (*10*), but our quantitative measurements indicate that foliar yellowing is delayed about 6 days (*11*). This report deals primarily with efforts to prevent monocarpic senescence by foliar applications of plant hormones.

Soybean plants [*Glycine max* (L.) Merrill cv. Anoka] were grown, and hormone solutions were sprayed onto the leaves (at 2-day intervals starting when the first flowers opened) as described (*9, 12*).

Foliar applications of cytokinin [6-benzyladenine (BA), kinetin, or diphenylurea] or auxin [NAA, but not indoleacetic acid (IAA)] delay the seed-induced foliar senescence in soybeans. However, these two hormones cause different changes (*4, 5, 13*). Cytokinins are more effective in preventing leaf yellowing with less retardation of abscission,

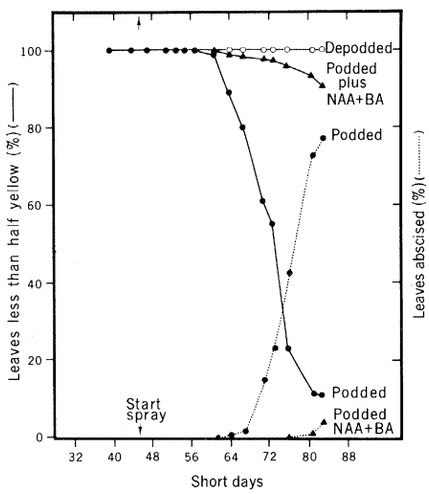

Fig. 1. Prevention of leaf yellowing and shedding during monocarpic senescence in attached soybean leaves by foliar applications of NAA and BA. These hormones (50 μ*M* in 0.05 percent, by volume, Tween 80) were sprayed on every other day. Scoring of leaf yellowing was done as described (*12*). The depodded plants showed no leaf abscission during this period.

Table 1. Effect of foliar applications of NAA and BA on seed yield,* number of seeds per plant, and number of pods per plant.

Item	Control	Treated with NAA + BA	Mean change (%)	Standard error of percent change
Seed yield*	6.6	6.0	−10	6
Seeds per plant	33	28	−10	6
Pods per plant	17	15	−14	8

*Grams per dry weight per plant.

and therefore there is a tendency to shed greenish leaves. In contrast, NAA retards yellowing less but abscission more, so that petioles and yellow leaves hang on longer than normal. Since these differences suggest that auxin and cytokinin work in different ways, we decided to apply them together. The combination of 50 μ*M* NAA and BA essentially suppresses leaf yellowing and abscission (Fig. 1). The slight senescence that does occur is in the lower leaves and is probably not monocarpic. Otherwise, the plants treated with NAA plus BA stay green long after the pods turn brown (at least 2 months beyond normal podded plants). The treatment with NAA plus BA produces no visible alterations in vegetative growth (such as stem elongation, leaf formation, and leaf expansion), which ceases soon after flowering starts.

It is important that the treatment with NAA plus BA does not retard fruit development, as measured by our sensitive numerical scoring system (*12*), nor does it change seed yields consistently. Paired *t*-test analyses suggest the treatments could produce a small decrease in seed dry weight per plant, in the number of seeds per plant, and in the number of pods per plant in this series of three experiments (five plants per treatment in each experiment) (Table 1); but the yield response has varied from a slight decrease to a slight promotion in earlier experiments. Although the treatment with NAA plus BA prevents senescence, it may fail to increase seed yields, pod number, or seed number because (i) the reproductive capacity of soybeans is fixed prior to foliar senescence (*3, 12, 14*) and (ii) the loss of capacity to produce reproductive structures may be induced by factors different from those that induce foliar senescence (and therefore are not altered by the same treatments).

Inasmuch as (i) the developing seeds normally obtain their carbohydrate, nitrogen, and certain other nutrients from the leaves and (ii) it has been supposed that this withdrawal may cause foliar senescence, it is important that the nitrogen and carbohydrate (starch) contents of the leaves treated with NAA plus BA do not decline during seed growth (Fig. 2). Since a plant that has been completely defoliated just prior to pod fill is unable to sustain filling of even a few pods (*6*), it follows that the seeds in the plants treated with NAA plus BA do not simply withdraw their needed nutrients from some reservoir other than the foliage [the major vegetative reservoir for nitrogen in soybean (*15*)]. Thus, treated plants have a much higher total nitrogen content and it is implicit that the treatment maintains the photosynthetic and nitrogen-assimilating capacity required to supply the seeds.

Whether the NAA + BA treatment corrects a hormonal deficiency or overrides a senescence-inducing hormone remains to be determined; however, the primary cause of monocarpic senescence of soybeans does not seem to be nutritional or hormonal deficiency (*3, 6, 16*), In any case, the hormone treatment provides another line of evidence, in addition to the surgical and genetic modifications (*6, 13, 17*), that soybean seeds can develop by drawing on the current production of assimilate in the plant and do

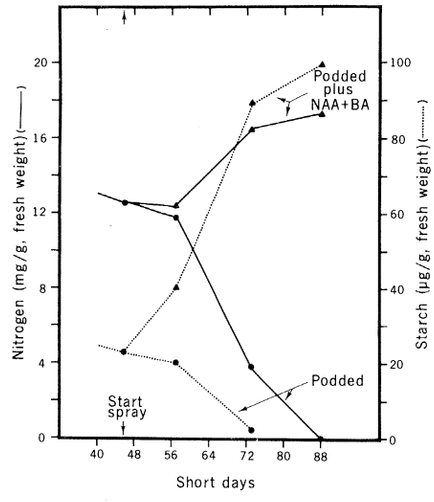

Fig. 2. Prevention of loss of nitrogen and starch from attached soybean leaves during monocarpic senescence by foliar applications of NAA and BA. Treatments were the same as described in the legend of Fig. 1. Prior to the nitrogen determination, the free BA and its glycosides (the main metabolites of BA), (*18*) were extracted with ethyl acetate after the leaves were thoroughly ground with water and the *p*H was adjusted to 8.0 with NaOH (*19*). This eliminates BA as a direct contributor of nitrogen. Total nitrogen in the extracted aqueous phase was determined as described (*15*). Iodine-precipitable starch was measured with anthrone reagent (*20*).

not have to mobilize the needed resources (for example, nitrogen) or divert needed nutrients from the leaves thereby killing the plant. The prevention of the internally programmed degeneration in soybeans through chemical treatment is not only of theoretical interest but may eventually open a way to yield improvement.

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Fluidity of the Rat Liver Microsomal Membrane: Increase at Birth

Abstract. *The lipid apparent microviscosity of the rat liver microsomal membrane on the first day after birth was found to be half of that observed on the last day of fetal life. This remarkable perinatal fluidization of the membrane resulted from a marked increase in the molar ratio of phospholipids to cholesterol.*

Among the dramatic events connected with the birth process there are sudden changes in the activity levels of many critical cellular proteins, such as enzymes and transport proteins. Studies in both laboratory animals and man have shown significant perinatal increases in the activity of some enzymes, while other enzymatic activities decrease from fetal life to adulthood (*1*). These developmental changes in enzyme activity are connected with changes in de novo protein synthesis. Since many of these enzymes are intimately associated with membranes it is to be expected that their activity will also depend on the physical properties of the membrane. A close dependence of uridine diphosphate glucuronosyltransferase (E.C. 2.4.1.17; UDPGT) activity on the fluidity of the lipids in guinea pig liver microsomal membranes has, in fact, been demonstrated by electron paramagnetic resonance analysis (*2*). It was therefore of interest to determine whether the perinatal changes in the activity of microsomal enzymes are accompanied by changes in membrane fluidity. We report here that at birth there occurs a remarkable increase in fluidity of the rat liver microsomal membrane.

Sabra rats were obtained from the Animal Facility of the Hebrew University and given free access to a diet of Purina Chow. Fetuses obtained from rats on the last day of pregnancy, and newborn rats on their first day of life, were decapitated and livers from each litter were excised and pooled together. Livers from adult rats (3 months of age) were examined individually. Homogenates (25 percent, weight to volume) were prepared in 1.15 percent KCl and microsomes were fractionated (*3*) and resuspended in 1.15 percent KCl. Microsomal protein was determined by the method of Lowry *et al.* (*4*). The apparent microviscosity ($\bar{\eta}$) of lipids was determined by measurement of fluorescence polarization with the use of the fluorophore 1,6-diphenyl-1,3,5-hexatriene (1 m*M* in tetrahydrofuran) (*5*). One microliter of this solution was added to 1 ml of a suspension containing 0.25 mg of microsomal protein and the mixture was incubated at room temperature for 1 hour. Fluorescence polarization was measured at 37°C by means of an instrument previously described (*6*). Excitation and emission wavelengths were 365 and 430 nm, respectively. Lipids were extracted from the microsomal membrane into a mixture of $CHCl_3$ and methanol (2 : 1 by volume) as described by Folch *et al.* (*7*). Total phospholipids were determined by phosphorus analysis (*8*) and cholesterol was determined by a cholesterol oxidase method (*9*).

The apparent microviscosity of the lipids of the rat liver microsomal membrane decreased by 48 percent between the last day of intrauterine life and the first day of extrauterine life (Table 1). A further small, but statistically significant, decrease in viscosity (15 percent) was observed at 3 months of age (Table 1). No significant differences in apparent microviscosity were found between male and female adult rats. The decrease in lipid apparent microviscosity observed in the perinatal period was paralleled by an increase in the microsomal phospholipid content of the liver while there was no change in its cholesterol content (Table 1). These results indicate that during the perinatal development of the smooth endoplasmic reticulum of rat liver, qualitative changes in lipid composition occurred which gave rise to increased phospholipid to cholesterol molar ratios

Table 1. Lipid composition and apparent microviscosity ($\bar{\eta}$) of rat liver microsomal membrane. Fetuses were studied on the last day of pregnancy and newborns during the 24-hour period following birth. Adult rats were 3 months old. Numbers in parentheses indicate number of litters, each litter consisting of 6 to 13 fetuses or newborn rats, or number of individual adult rats. The $\bar{\eta}$ values are expressed as means ± standard error. Protein, phospholipid, and cholesterol content were determined in composite pools from all the microsomal preparations used for measurement of $\bar{\eta}$ in each of the three groups.

Microsomes	$\bar{\eta}$ (poise)	Protein (mg/g liver)	Phospholipid (μmole/g liver)	Cholesterol (μmole/g liver)	Phospholipid/cholesterol molar ratio
Fetus	2.33 ± 0.13* (9)	14.0	3.86	1.12	3.45
Newborn	1.22 ± 0.05*† (7)	20.9	6.05	1.14	5.31
Adult	1.04 ± 0.02† (21)	22.3	8.05	1.07	7.52

*Significant at $P < .001$. †Significant at $P < .005$.

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