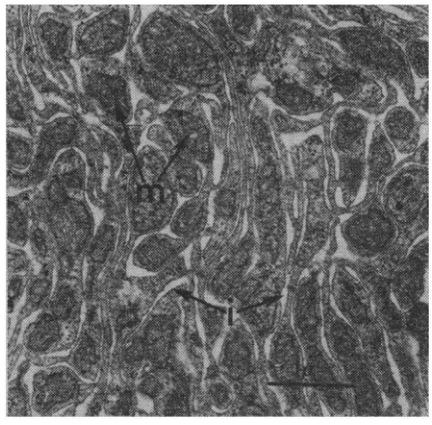

Fig. 5. Electron micrograph of a cell from a labial gland of *Antheraea pernyi* fixed shortly before adult ecdysis (× 11,500). The cell has been sectioned obliquely and the apical side, closest to the lumen, is pictured here. The surface of the cell is extensively infolded so that the apical region appears as a series of irregular, interconnected plates of cytoplasm surrounded by narrow, clear clefts of the extracellular spaces (*i*). Numerous mitochondria (*m*) are seen in the cytoplasmic plates. Fixed first in glutaraldehyde and then in 1 percent osmium tetroxide, and stained with lead citrate and uranyl acetate.

(approximately 20 meq/liter) and in isotopic experiments appears to exchange rapidly with blood chloride. Phosphate is not detectable. A substantial *p*H gradient exists across the walls of the gland (blood, about 6.5; secretion, about 8.5).

The capacity of the lumen is only a small fraction of the volume of the solvent secreted before the moth escapes from its cocoon; most of the secretion must be elaborated during a relatively brief period. The expected changes in hemolymph composition (decrease in potassium, increase in the excluded ions) are indeed observed during this interval. Our measurements suggest an active secretion of K^+ into the lumina of the glands, followed by the passive flow of water and anions (at least Cl^-). Consistent with this interpretation are determinations of electrical potentials across the secretory cells, the lumen being positive both in vivo and in vitro (*11*).

The proteinase is secreted first and coats the maxillary appendages as a dry incrustation. Then, after the moth has forced its head from the pupal exuviae, the solvent is ejected from the facial aperture between the maxillae. The first drops dissolve the enzyme and bring the latter into contact with the overlying cocoon. Subsequent secretion promotes enzymic attack on the cocoon in two ways: it wets the extensive area which must be cleared of sericin, and it serves to maintain the enzyme at optimum *p*H (approximately 8.5). Although the buffering capacity of bicarbonate is poor in this range, the fluid continuously neutralizes the carboxylic groups liberated during sericin digestion without endangering the optimization of the reaction by excessive rise in *p*H. In this sense, the labial glands resemble a *p*H-stat, continuously titrating the reaction to an end point of about *p*H 8.5.

In establishing an escape-hatch in the cocoon, the enzyme's natural substrate is the interstitial sericin which binds the fibroin filaments. We are reluctant, however, to imply limitation of its potency by adopting the name "sericinase": the enzyme is both a powerful proteinase, active on all soluble proteins so far tested, and a trypsin-like esterase. In recognition of its unusual source and remarkable biological role, we propose to call it "cocoonase."

It may be noted that the enzyme is not elaborated by *Hyalophora cecropia* or *Rothschildia orizaba*, two silkworm species whose cocoons are equipped with prominent, preformed valves. Indeed throughout the New World, Saturniidae unsealed cocoons correlate with truly vestigial maxillary galeae (*12*). However, despite the absence of cocoonase, these two species, the only ones examined thus far, can secrete a voluminous liquid which is entirely derived from the labial glands. *Samia cynthia*, which spins a cocoon with a particularly tight valve, is an intermediate form in that the galeae, though smaller than those of *A. pernyi*, are not vestigial; a small amount of cocoonase is synthesized.

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Plant Damage Caused by Irradiation of Aldehydes

Abstract. *The report that damage to petunia has been correlated with the presence of aldehydes in the atmosphere is discussed in relation to recent laboratory findings. Laboratory investigations have shown that irradiation of formaldehyde in air will not cause plant damage to the varieties of petunia, pinto bean, and tobacco wrapper used, even when nitrogen oxide is added to the system. Irradiation of propionaldehyde in air does cause damage to these plants. Addition of nitrogen oxide to the irradiated propionaldehyde-in-air system does not markedly increase damage.*

Injury to leaves of Snowstorm petunias has been associated with atmospheric levels of aldehydes (*1*). The injury is characterized by necrotic banding of the upper leaf surface and glazing of the lower leaf surface. Aldehydes were determined chemically by the bisulfite method, and plant damage occurred within 2 days after the atmospheric aldehyde content exceeded 0.20 parts per million.

Stephens and co-workers (*2*) photooxidized several aldehydes in air and concluded that the products of certain aldehydes would damage the lower leaves of pinto bean and also would damage petunia. The role of added nitrogen oxides was not investigated in this earlier work. Small concentrations of nitrogen oxides may have been present in the mixtures.

We have obtained data on the irradiation of formaldehyde and propionaldehyde in the presence of low concentrations of nitrogen oxides and in the presence of added amounts of nitrogen oxides. Composition of the mixtures, oxidant levels, and intensities of plant

damage are listed in Table 1. The exposures were carried out under fluorescent lamp illumination in a plant chamber through which the irradiated mixture from a large dynamic flow reactor was passed for a 4-hour period.

Pinto bean (*Phaseolus vulgaris* L. var. pinto), tobacco wrapper C (*Nicotiana tabacum* L. var. Bel. C), tobacco Smyrna (*Nicotiana tabacum* L. var. Smyrna), and petunia (*Petunia hybrida* Vilm. var. Celestial Rose) were selected for this study because they develop identifiable symptoms that can be attributed to specific phytotoxicants produced in irradiated auto exhaust (*3*).

Plants were selected for fumigation at specific stages of growth rather than by chronological age from date of planting. Pinto beans were fumigated at two growth stages. The first stage was just before the first trifoliate bud began to open; the second stage was when the primary leaf was fully expanded at the time of unfolding of the second trifoliate bud. Tobacco wrapper C and tobacco Smyrna were selected when the plant had a single stalk with four to six fully expanded leaves. Petunia plants were selected before the flower bud began to open.

The index used for evaluation of damage is based on a scale of 0 to 4. An index of 4 corresponds to complete damage to sensitive tissue.

The results in Table 1 clearly show that formaldehyde caused no plant damage whether in the presence of 0.05 or 0.9 ppm of added nitrogen oxide. No damage occurred to any of the species investigated, including tobacco wrapper, despite the presence of appreciable concentrations of oxidizing substances, which may be mostly organic oxidants rather than ozone. The lack of damage in the presence of irradiated formaldehyde-in-air mixtures confirms the previous work and shows that the addition of nitrogen oxide will not result in formation of a phytotoxicant.

Irradiation of propionaldehyde in air definitely will cause appreciable plant damage to tobacco wrapper, pinto bean leaves at various stages of development, and petunia. Phytotoxicants are formed both at low nitrogen oxide concentrations and in the presence of added nitrogen oxide. The degree of plant damage is not directly related to nitrogen oxide concentration and possibly not directly to peroxyacyl nitrate concentration.

More detailed observations indicate five classes of injury based on response patterns for tobacco, pinto bean, and petunia plants exposed to irradiated propionaldehyde. These same response patterns were observed previously when plant varieties were exposed to irradiated automobile exhaust (*3*). Irradiated auto exhaust rarely injured old primary leaves of pinto bean severely.

Table 1. Plant damage from the irradiation of aldehyde and mixtures of aldehyde and nitrogen oxide.

Concentration (ppm)					Av. index	Degree of damage*				
Propionaldehyde	Formaldehyde	NO_x	NO_2 peak	Av. oxidant†		Class 1 (young leaves)	Class 2 (pinto bean)	Class 3 (tobacco and petunia)	Class 4 (tobacco and petunia)	Class 5 (pinto bean)
3.4		0.1	0.15	0.1	2.0	xx	xxx	xx	xx	xxx
3.5		0.9	0.7	0.3	2.6	xx	xxx	xx	xx	xxx
0.52		0.5	0.5	0.05	1.5	tr	xxx	x	x	xxx
	5.6	0.05	0.1	0.25	0.0	0	0	0	0	0
	6.1	0.9	0.6	0.65	0.0	0	0	0	0	0

* Degree of damage: Trace, tr; little, x; moderate, xx; heavy, xxx. † The readings from a Mast Oxidant Instrument were averaged over the 4-hour exposure period with correction for nitrogen dioxide. Multiplication of these values by a factor of 1.5 usually resulted in concentrations in reasonable agreement with those obtained by colorimetric analyses.

Class 1 involves the palisade cells of young leaves. Initially a waterlogged appearance develops on the upper surface of young leaves of tobacco, pinto bean, and petunia. Class 2 injury produces glazing on the lower surface of young and trifoliate leaves of pinto bean and on young tobacco leaves. Classes 3 and 4 affect the palisade cells; class 3 affects the newly expanded leaf (*4*); class 4 affects the old leaf. Tobacco wrapper C is a good indicator of both classes of injury. Class 5 injury appears as dehydrated patches and areas on the upper surface on old primary leaves of pinto bean. This injury resembled severe sun scald on the upper surface of the leaves. The injury was severe.

Class 2 injury resembles damage caused by the PAN (peroxyacyl nitrate) series of compounds, whereas class 3 injury has been related to ozone. The heavy damage to young primary and trifoliate leaves of pinto bean caused by irradiating propionaldehyde even in the presence of low concentrations of nitrogen oxide may indicate the presence of another type of phytotoxicant in addition to peroxyacyl nitrates.

Although Brennan *et al.* (*1*) observed lower-surface damage to "Snowdrop" petunia leaves, only upper-surface damage to "Celestial Rose" petunia was observed in the present study with irradiated propionaldehyde mixtures. Lower-surface glaze, however, was seen in young primary and trifoliate leaves of pinto bean plant.

In the work by Stephens and coworkers (*2*) a mixture of irradiated cis-2-butene and ozone caused no plant damage. Since acetaldehyde was formed as a product in high yield, one can assume that acetaldehyde does not cause significant plant damage when irradiated. In other experiments in our laboratories, no plant damage was observed from irradiated mixtures of 1,3-butadiene and nitrogen oxide. Since acrolein was formed at 1 ppm as a product, it can be assumed that acrolein does not cause significant plant damage when irradiated to the varieties of petunia, pinto bean, and tobacco wrapper being studied. Although further irradiation work with a complete series of aldehydes is needed, it appears probable that irradiations of only propionaldehyde and higher-molecular-weight saturated aldehydes cause plant damage. Nitrogen oxides when irradiated with such olefins as 1-butene, 1-pentene, 2-pentene, 1-hexene, 2-hexene, and 3-hexene will produce propionaldehyde and other higher-molecular-weight saturated aldehydes. The role of such aldehydes in causing the plant damage patterns produced by irradiating mixtures of olefins and nitrogen oxide is of interest.

The laboratory work suggests several comments with respect to the atmospheric observations of Brennan *et al.* (*1*). The bisulfite method used by these investigators, as they were aware, responds to both aldehydes and ketones. The ketones of low molecular weight, acetone and methylethyl ketone, are formed from the photooxidation of various olefins including isobutene, 2-methyl-2-butene, 2-methyl-1-butene, 2-methyl-2-pentene, and 3-methyl-2-pentene. Acetone and methylethyl ketone show very low reactivity in photooxidation reactions (*5*). Formaldehyde con-

stitutes approximately half the aliphatic aldehyde present in auto exhaust and atmospheric aldehydes (*6*). Consequently, the propionaldehyde and higher-molecular-weight aldehydes make up only a small portion of the atmospheric aldehydes and ketons analyzed by the bisulfite method. The limit of 0.20 ppm given by Brennan *et al.* (*1*) may have little relation to the actual atmospheric concentration of aldehydes that form phytotoxicants. It does not follow a priori that these particular aldehydes are linearly related to the analytical measurement obtained by the bisulfite method. The more specific analytical techniques available for aldehydes (*6*) are preferable.

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Spore Discharge Mechanism in Basidiomycetes

Abstract. *Spore discharge in basidiomycetes is effected primarily by the explosion of a small gas bubble in the area of the apiculus of the spore and by pressure of residual gas (probably carbon dioxide) that has accumulated between the inner wall and outer membrane of the spore apparatus. A somewhat similar mechanism of discharge has been discovered in an undescribed mycetozoan.*

Ever since the discovery (*1*) that the basidiospores of club fungi are discharged forcibly from the sterigmata bearing them, there have been various theories attempting to explain the phenomenon. Both Ingold (*2*) and Prince (*3*) have summarized these theories and have presented their own. Almost all the hypotheses have been based on the idea of discharge resulting from increased turgor within the spore apparatus to a point at which the wall in the narrow region connecting spore with sterigma is ruptured and the pressure causes the spore to be shot away. Fayod (*4*) was probably the first to note that a droplet appears at the apiculus near the base of the basidiospore just before discharge, and Buller (*5*) elaborated upon this in his extended observations.

Because Buller studied the process more extensively than any other investigator, the so-called "droplet mechanism" of spore discharge came to be associated primarily with his name. Although Buller thought that the droplet had something to do with spore discharge, neither he nor any other investigator could satisfactorily explain how a liquid droplet could be exuded from the spore apparatus at the very moment when the greatest amount of pressure is needed within for spore discharge. Buller also concluded that the droplet was discharged along with the spore, but this idea was supported by tenuous circumstantial evidence.

Recently, during studies of a new and as yet undescribed mycetozoan, I observed that the single spore is shot away forcibly from the short stalk bearing it. Upon closer observation I noted that, just before discharge, a gas bubble, apparently arising from an accumulation of a gaseous layer between inner spore wall and an outer membrane, develops on one side of the spore. After a short interval, the bubble bursts, and this minute explosion appears to be primarily responsible for dislodging the spore from its stalk and generally propelling it for some distance across the surface of the agar medium. The gaseous nature of the bubble (probably CO_2 of metabolism) was verified by the observation that it sometimes bursts without effecting discharge of the spore. Occasionally, bubbles appear and burst repeatedly without causing discharge (*6*).

Since spore discharge in basidiomycetes bears considerable resemblance to that of the new mycetozoan, observations were accordingly begun to determine whether a similar mechanism might be operable in this large group of higher fungi. Such is the case. Spore discharge was examined in the basidiomycetous yeast, *Sporobolomyces*, which was also studied extensively by Buller, and in several agarics. *Sporobolomyces* sp. was observed both in agar plates and on thin agar drops inverted on cover slips in van Tieghem cells. The medium used was Difco cornmeal-dextrose agar supplemented with 0.1 percent yeast extract.

Sporobolomyces reproduces both by budding and by the production of ballistospores. In the latter, a cell produces a sporogenous hypha which extends into the air from the agar surface and produces from one to several ballistospores (successively) on tapered extensions or sterigmata from the upper part of the hypha. Both the hypha (including sterigma) and spore assume an opacity that is due at least partly to the accumulation of a gas, again probably CO_2 of metabolism, between an inner wall and an outer, rather pliable, membrane (Fig. 1). The wall and membrane of sporogenous hypha and spore are continuous. Where the hypha is in contact with the agar or is submerged in the film of water that often collects on the agar surface in van Tieghem cells, there is no accumulation of gas and the outer membrane is not readily discernible. However, where the agar surface is dry, that part of a sporogenous hypha which sometimes lies on the surface of the agar may show an accumulation of a gaseous layer between inner hyphal wall and outer membrane on the side not in contact with the agar.

Just prior to spore discharge there no longer appears to be any connection between the inner walls of sterigma and spore. The mature spore is asymmetrically inclined away from the apicular side. There is a small but distinct pocket of gas in the region of the apiculus. At the time of discharge the outer membrane in the apicular region, which is probably its weakest spot, suddenly expands under pressure from the gas and enlarges over a period of several seconds, or sometimes longer, then bursts. This small explosion, in addition to some residual pressure in the remaining gas layer, is apparently the main force that ruptures the outer membrane just below the spore and propels the spore from its sterigma. The asymmetrical position of the spore would, of course, assist in unbalancing it in the direction of the force exerted by the bursting bubble.

When a micromanipulator needle is touched to attached spores that appear mature, some of these seem to become dislodged partly by force from within