

# 孟繁静论文选集

中国农业大学生物学院  
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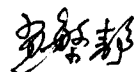
王静如

## 前 言

我即将退休之际，我的学生们出于对我真挚的爱护和尊重，建议将近十多年来我科研组的论文汇编成册作为纪念。它即是本研究工作的记实，又体现了科研组成员间互相友爱，拼搏攻关的团结气氛。

在李季伦教授的启发、鼓励和帮助下，于70年代末开始立题，对“高等植物的玉米赤霉烯酮”进行了系统研究，并连续获得国家自然科学基金的资助，工作得以持续并取得初步成果。当前生命科学的发展日新月异，希望在新形势下，本研究将有更为丰富和新颖的内容，为我国的科技事业添砖加瓦。

我们衷心感谢娄成后教授、曹宗巽教授和闫龙飞教授始终对我们工作的爱护和指导，感谢校各级领导和植物生理组的老师们和学生们的支持。



谨以此书献给孟繁静教授七十寿辰暨  
从事植物生理研究教学四十七周年

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# Uninfected Cells in Ureide- and Amide-exporting Legume Root Nodules

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Legumes are divisible into various groups based on the major nitrogenous compounds transported from the nodules to the shoots [1]. For example, pea, vetch and lupine belong to the amide exporters, a group that transports fixed nitrogen mainly as asparagine. Soybean, bean and cowpea, on the other hand, belong to the ureide exporters, a group in which the ureides, allantoin and allantoic acid, are major products of nitrogen assimilation in the nodules and the principal form of transport of nitrogen to the shoots.

Although there are numerous developmental and structural differences between the nodules of the amide and ureide exporters [2], it is a characteristic of both groups and of the nodules of most legumes that many of the cells in the central infected region of the nodule remain uninfected throughout nodule life [3].

It is only in the last 3-4 years that attention has been directed to these uninfected cells. Apparently it had been tacitly assumed that having escaped infection, they play an insignificant role in the metabolism of recently fixed nitrogen, and simply provide supportive functions in the nodule. We now know, however, that in the ureide exporters, the uninfected cells have a special role to play in production of the export products. In preparation for this they undergo a highly specialized ultrastructural differentiation not shown by the infected cells, namely, the development of numerous enlarged peroxisomes (Fig. 1) and an extensively ramifying tubular endoplasmic reticulum [4,5].

The pathway of ureide production in two ureide transporters, bean and cowpea, has been examined recently in several laboratories [6-11]. These studies are in agreement that the ureides are produced in the nodules from purines, which in turn arise from amino acids that incorporate ammonia produced from  $N_2$  by the bacteroids.

Hanks and coworkers [12] have shown for soybean, and Shelp and coworkers [13] for cowpea, that the early steps in ureide production occur in the infected cells, while the conversion of uric acid to allantoin occurs in the peroxisomes of the uninfected cells. The conversion of allantoin to allantoic acid apparently takes place in the endoplasmic reticulum [12]. Triplett [14] has recently shown histochemically that xanthine dehydrogenase is located in the infected cells, implying that the purines and uric acid are made in the infected cells and uric acid travels to the uninfected cells. From the uninfected cells the ureides move into the vascular bundles in the nodule cortex, and then to the upper parts of the plant by way of the xylem stream.

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NITROGEN FIXATION AND  $CO_2$  METABOLISM

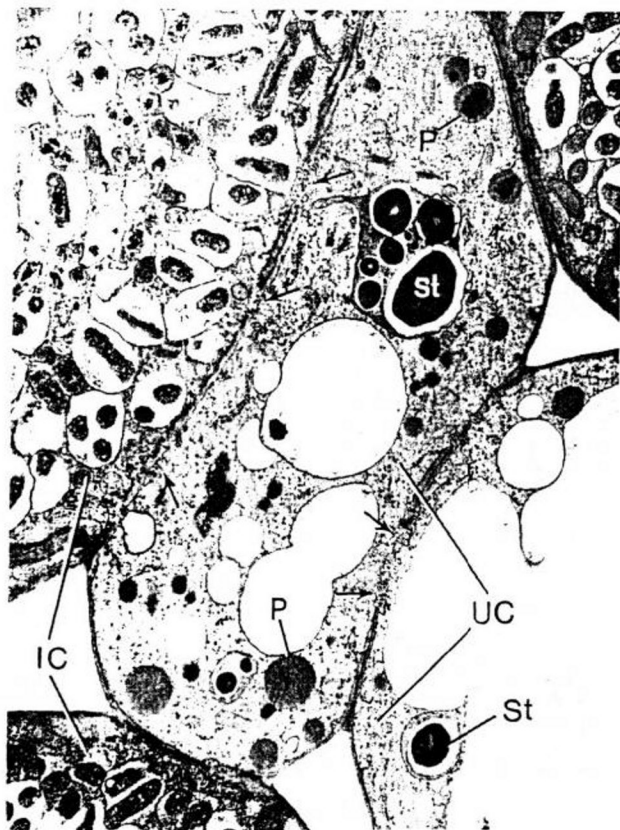


Fig. 1. Electron micrograph of the infected region of a nodule of soybean 4 weeks after inoculation, showing uninfected cells contiguous with infected cells. UC, uninfected cell; IC, infected cell; P, peroxisome; SR, starch in plastid. Note numerous plasmodesmata (arrows) between an infected and an uninfected cell, and also between two uninfected cells. X6600

Thus there is a degree of structural and functional specialization in the central tissue of the soybean nodule: a division of labor in which the uninfected cells function as essential components in the symbiotic relationship between host and bacterium. Maintenance of a reasonable balance between the two types of cells would seem to be essential for effective nodule functioning, since if most or all of the cells in the central region were to become infected, conversion of amino acids to the ureides would be inefficient or non-existent.

Furthermore, the distribution of uninfected cells among the infected cells may be regulated so as to be appropriate for the efficient metabolism of nitrogen products. Processing of recently fixed nitrogen in the nodule thus seems to depend on differentiation at two levels higher than the biochemical: 1) the cellular level, entailing differentiation of the necessary organelles in the uninfected cells, and 2) the organ level, including the establishment of infected and uninfected cells in the proper number and distribution with respect to one another.

Our purpose in the current work is to characterize those features of mature nodules which provide evidence bearing on the types of controls in effect during development of uninfected cells. First, some quantitative information on the differences in organelle volume and number between uninfected and infected cells of soybean nodules will define the extent of the ultrastructural divergence that develops for three key organelles: mitochondria, plastids and peroxisomes [5].

Ten micrographs from each of 10 nodules, each micrograph containing similar cytoplasmic areas of the two cell types, were taken at the same magnification. From prints of these we obtained the number of profiles of the three organelles, and the total area of the cytoplasm and of each organelle.

The results [5] are shown considerably condensed in Table I. They did not entirely conform to our intuitive expectations. As might have been expected from the enormous demand of the bacteroids for ATP, the mitochondria in the infected cells have over 4X the volume and over 3X the number of profiles per unit cytoplasm compared to those in the uninfected cells.

It is interesting that the plastids occupy about the same volume per unit cytoplasm. The observations are suggestive in considering the biochemical results of Hanks et al. [12] demonstrating that several enzymes involved in purine synthesis are active in plastids from both cell types. It is questionable whether the plastids of the uninfected cells are producing purines for ureide production, in view of Triplett's results on xanthine dehydrogenase [14].

Although there are nearly 6X as many plastid profiles per unit cytoplasm in the infected cells, it cannot be concluded that there are differences in plastid numbers, because the organelle is so variable in shape. Serial

sections reveal that the plastids in the infected cells are vermiform, so that a single organelle can undulate in and out of the plane of section and be counted several times.

TABLE I. Statistical Data\* on Three Organelles of Uninfected (U) and Infected (I) Cells in the Infected Region of Soybean Root Nodules.

Quantity measured	Mitochondria		Plastids		Peroxisomes	
	U	I	U	I	U	I
Total area as % of cytoplasmic area	1.81	7.72	<u>6.31</u>	<u>5.71</u>	3.65	0.06
No. profiles per 100 $\mu\text{m}^2$ cytoplasm	6.2	20.1	1.6	9.3	<u>6.6</u>	<u>4.1</u>

\*Numbers connected by underlining are not significantly different.

Large peroxisomes are characteristic of the uninfected cells. However, small peroxisome-like bodies do occur in the peripheral cytoplasm of the infected cells. The total volume of the peroxisomes is 60-fold greater in the uninfected cells (Table I). In comparing numbers of peroxisomes, it is necessary to correct for overcounting larger organelles, since larger spherical organelles are overcounted in sections in proportion to their diameters. When this is done, then there is no significant difference in numbers of peroxisomes per unit cytoplasm between the two types of cells. Apparently the organelle proliferates in the infected cells in the early stages of infection, but fails to develop in these cells.

The ultrastructure of two additional ureide exporters, bean and cowpea, resembles that of soybean in the development of enlarged peroxisomes and tubular ER in the uninfected cells.

In the uninfected cells of the amide exporters pea, vetch and alfalfa, the differentiation found in the ureide exporters is absent. The cytoplasm of these cells has no distinctive features that set it apart from the cytoplasm generally characteristic of parenchymatous cells.

A large amount of starch is frequently seen in the infected cell plastids adjacent to the intercellular spaces in both vetch and alfalfa. The starch sheath may completely border the cytoplasm of the infected cells.

An even more interesting phenomenon is that where mitochondria and plastids occur side by side in the peripheral cytoplasm of infected cells, the plastids are always external to the mitochondria, that is, next to the plasmalemma. This is true in both vetch and alfalfa. In the ureide transporters, however, although plastids and mitochondria are also located at the periphery of infected cells, they do not show such stratification.

In view of the essentiality of the uninfected cells in the ureide exporters, the abundance in these cells of several key organelles, and the strategic position of the cells in the metabolic flow of fixed nitrogen, we felt that their developmental and structural relationships, both to the infected cells and to the nodule as a whole, should be examined.

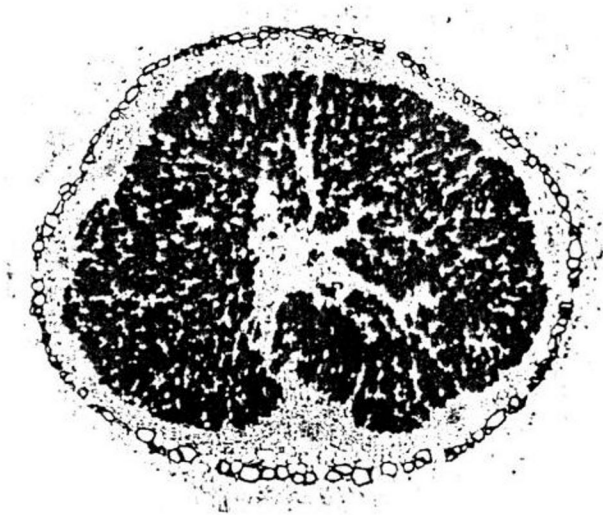


Fig. 2. Longitudinal section of a mature soybean root nodule. The vacuolate uninfected cells are visible among the darkly stained infected cells. Some of the uninfected cells are loosely organized into rays running through the infected tissue. X45

The allocation of space and cell numbers to the two cell types has been analyzed using standard stereological methods [15-18]. The nodules came from plants grown in a mixture of perlite and vermiculite and fertilized with a modified Hoagland's solution without nitrogen. The ratio of cell numbers was calculated from a formula of Pugh and coworkers [18], which corrects for the effect of the size difference between infected and uninfected cells on the sampling frequency.

Casual inspection of sections of the soybean nodule, (Fig. 2) suggests that the infected cells outnumber the uninfected cells. At any rate, the volume occupied by infected cells is obviously greater than that of uninfected cells.

Table II gives the results of the stereological study. As expected, the infected cells in soybean without nitrogen do have a greater volume than the uninfected cells. This volume ratio of 1:3.7 corresponds to 78% of the infected region being occupied by infected cells.

TABLE II. Stereological Data on Cell Types in the Infected Region of Root Nodules of Alfalfa Compared with Soybean

Legume species	Nutrient medium	Ratio Uninfected : Infected	
		Volume	No. of cells
Soybean	-N	1 : 3.7	1.6 : 1
	+NO <sub>3</sub> <sup>-</sup>	1 : 2.2	2.1 : 1
Alfalfa	-N	1 : 3.7	1.3 : 1

But contrary to our expectation, in soybean the uninfected cells outnumber the infected ones by a ratio of 1.6 to 1. This result indicating a 60% excess of uninfected cells is compatible with the volume data because the infected cells are larger, having on the average 4X the diameter of the uninfected cells. The volume results agree with those reported by Bergersen [19].

We do not yet understand what controls whether a cell will become infected or not, and what proportion of the total number of cells in the infected region will become infected. Considering the necessity of metabolic interaction between the two types of cells in ureide transporters, it would seem likely that there is an optimal

ratio between the two under a given set of environmental conditions. It would be worthwhile to know whether this ratio is relatively insensitive to, or highly dependent upon, environmental conditions.

As one approach to this question, we have employed a considerable environmental modification, that is, the inclusion of 7.5 mM nitrate nitrogen in the nutrient medium with which we watered the plants. This modification resulted in fewer nodules per plant and slower nodule growth.

As shown in Table II, the organization of the infected region of the nodule was also somewhat changed by the nitrate treatment, that is, the uninfected cells occupied a larger percentage of the volume of the infected region. Also, they occurred in even greater numbers relative to the infected cells - a ratio of more than 2 uninfected cells to each infected cell - than in the plants without nitrogen. The volume ratio of 1:2.2 corresponds to a volume fraction of 69% for the infected cells. Ralston and Imsande [20] have reported that the early stages in the process of nodule development are the most sensitive to nitrate. Possibly nitrate affects growth of the infection thread or release of bacteria from the thread, causing somewhat fewer cells to become infected.

Another way of examining the relationship between the two cell types is to inquire into their proximity to one another. Bergersen [19] has found that infected cells have on the average at least 20% of their surface area in contact with uninfected cells. This suggests that every infected cell may be in direct contact with at least one uninfected cell. However, his data are also consistent with a structure in which some infected cells are separated by one or more cell widths from the nearest uninfected cell. We have randomly sampled infected cells from 10 nodules and used the statistics of Bernoulli random variables to define the expected range for the percentage of infected cells which directly touch at least one uninfected cell. Sampled cells which were not in contact with an uninfected cell in the plane of the sampled section were traced through adjacent serial sections to look for sites of contact.

As shown in Table III, we found all sampled infected cells in soybean to be in contact with at least one uninfected cell. This allows us to conclude that the percentage of infected cells in contact with uninfected cells is, if not 100%, then very close to 100%. The range of values more probable than 5 out of 100 is given as the 95% confidence interval in the table.

We know also from electron micrographs that plasmodesmata are abundant between contiguous infected and uninfected cells (Fig. 1). It is not difficult to rationalize the functional economy of having the two interacting cells in contact, but how this relationship is achieved during the early days of nodule development remains an important unsolved problem. One clue may be found in our observation of cross sections of infection threads in fully

differentiated uninfected cells which are devoid of any released bacteroids. We have never, in fact, observed released bacteroids in recognizably differentiated uninfected cells. Could it be that release of bacteroids from the infection thread is inhibited by a cell if its differentiation as an uninfected cell proceeds beyond a certain stage? Or that release of bacteroids is inhibited if too many cells in contact with the given cell already have released bacteroids?

TABLE III. What Proportion of the Infected Cells is in Contact with at least One Uninfected Cell?

No. of infected cells sampled	No. of infected cells in contact	No. of infected cells not in contact	No. of cells followed out of plane of section	95% confidence interval for % of infected cells in contact*
Soybean: 1000	1000	0	37	99.7 - 100
Alfalfa: 900	900	0	41	99.6 - 100

\*95% confidence interval based on binomial distribution for a Bernoulli random variable.

As shown in Fig. 2, the uninfected cells are not distributed evenly throughout the nodule. The central part of the infected region has the greatest number of uninfected cells per unit area. In the middle and outer parts of the infected region, some of the uninfected cells are loosely organized into rays, as first reported by Bergersen and Goodchild [21]. Our observations of a three dimensional reconstruction of a nodule and of the surface of the infected region of several nodules have shown that the infected region is irregularly divided into complete or incomplete compartments by partitions composed of uninfected cells.

We have noted infections developing from the invasion of separate root hairs, and believe that the wider partitions divide aggregates which have arisen in this way. For example, a single nodule may have a partition of uninfected cells dividing it completely in half.

Incomplete partitions within the infected region may be numerous or few. In early emergent nodules, partitions of different widths are seen between aggregates of cells with dense cytoplasm. These partitions probably persist to produce the divisions seen in mature nodules. Within the partitions in mature nodules, long and narrow uninfected cells are often found, suggesting that the partitions may serve as avenues for transport within the infected region.

Finally, to what extent do our findings on soybean apply to the amide exporters? Firstly, there is no evidence at present that, in the amide exporters, the uninfected cells participate in producing the export products of fixed nitrogen as they do in the ureide exporters. Consistent with this is the absence of any special ultrastructural features of these cells in the amide exporters so far examined by us, namely, pea, vetch and alfalfa.

In view of this, data on the relative volumes occupied by the two cell types in the infected region of alfalfa, and the relative numbers of cells as well, are rather surprising, as seen in Table II. The volume ratio is the same as for soybean. In both cases, the infected cells occupy nearly 80% of the total volume of the infected region, and the uninfected cells only a little over 20%. Also, as in soybean, the uninfected cells in alfalfa outnumber the infected, but, under similar environmental conditions, by 30 rather than 60%.

Perhaps even more surprising are the results of Table III, showing that just as in soybean, in alfalfa every infected cell is in contact with at least one uninfected cell.

For the ureide exporters, the segregation of the terminal steps in ureide production in uninfected cells finds a plausible rationale in the low affinity of uricase for oxygen, and the greater availability of oxygen in the uninfected cells than in cells filled with oxygen-demanding bacteroids.

To date we know of no essential metabolic interactions between infected and uninfected cells of amide exporters, but the similarity of alfalfa to soybean, both in the plurality of uninfected over infected cells and the contact with uninfected cells made by all the infected cells, suggest that in the amide exporters, also, we should be on the alert for some meaningful biochemical interplay between the two cell types.

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# 玉米赤霉烯酮的研究

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## 提 要

自京郊分离的玉米赤霉菌 (*Fusarium roseum graminearum*) BAU-z8 菌系的培养物 (大米培养基) 中提出了一种具有动物雌性激素作用的物质。根据其熔点、紫外和红外光谱等理化性质, 被鉴定为玉米赤霉烯酮 (zearalenone)。一公斤大米制备的培养基经接种发酵后, 可提出1.4克的玉米赤霉烯酮结晶。本文报导了玉米赤霉菌的选育和培养, 玉米赤霉烯酮的提取、纯化的方法, 以及有关玉米赤霉烯酮的生物学效应的研究。

试验表明, 玉米赤霉烯酮可显著刺激小白鼠子宫增重和促进北京鸭生长。幼令小白鼠经用玉米赤霉烯酮油剂皮下注射, 三天后剖检, 其子宫鲜重可达对照者4—5倍, 甚至7倍。北京鸭于填鸭开始时, 每日口服含0.7mg玉米赤霉烯酮胶囊, 10天内其平均体重比对照者增约16.5% ( $P < 0.01$ ) 未发现副作用。

值得注意的是, 我们发现已渡过春化的冬小麦生长锥中也存在类似玉米赤霉烯酮的物质, 其生长锥的乙酸乙酯抽提液经薄板层析后, 在硅胶薄板层析谱上有和玉米赤霉烯酮相同 $R_f$ 值的兰荧光斑点, 并可被 $FeCl_3$ 显紫红色。可是经含100ppm玉米赤霉烯酮的 $NaHCO_3$ 水溶液浸种处理的冬小麦, 于常温、长光照下分别播种于温室和田间后, 仅观察到生长锥分化到二棱期, 未见抽穗。看来玉米赤霉烯酮尚不能完全代替低温春化作用。另一方面, 经浸种处理的墨西哥春小麦 (Potam 品种), 在春季田间播种后, 不但生长良好, 而且比对照提前抽穗5天左右。关于玉米赤霉烯酮对小麦生长发育的效应的研究仍在进行中。

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\* 李秀玉同志参加过菌种分离工作