decanted, and the cells were fixed in 2.5% buffered glutaraldehyde for 2 h at room temperature (Buffer solution: 0.15 M NaCl, 0.05 Tris, pH 7.0, 0,5 mg/ml Carbowax, passed through a 0.45 µm Millipore filter). After 3 washes of the fixed cells in buffer, 1.0 colloidal gold + con-A conjugate was added to 0.4 ml cell sediment and the system was incubated at room temperature for 10 h. Subsequent addition of 3 ml buffer, mild shaking and hand centrifugation was followed by 2 washes in buffer solution, dehydration in amylacetate by addition of $CO_2$ at the critical point, drying, and gold evaporation. The preparations were examined in a Jeol 100 C Temiscan electron microscope. The control preparations were dehydrated immediately after fixation, and processed further as above.

The binding of con-A by *Tetrahymena* was indicated by the presence of many colloidal gold particles on the body ciliature of conjugate-treated cells, contrasted to absence of the tracer in both membrane and ciliary regions of controls. Although in the treated preparations the colloidal particles could be seen along the entire length of the cilia, the bulk was obviously associated with the tips of cilia, and there was practically no indication of lectine binding by the cell membrane and oral ciliature.

This experimental observation has substantiated the former implication[11,12] that unicellulars, including *Tetrahymena*, do possess lectine binding sites, and it also throws light on the nonuniform distribution, i.e. absence of lectine-binding in certain cell regions. Absence of binding along the cell mebrane of the body, despite its presence along the cilia, suggests the specialization of certain cell membrane regions. The apparent lack of binding sites in the oral structures, contrasted with their abundance at the ciliary tips, supports the same conclusion. There is reason to postulate that, in unicellulars, certain sites of chemical recognition (receptors) are localized in the body ciliature predominantly on the tips of cilia, which seem to maintain functional superiority also in this respect. Evidence of cell membrane specialization at a relatively very low phylogenetic level may promote the understanding of receptor specialization at higher levels.

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## Pinitol, a larval growth inhibitor for *Heliothis zea* in soybeans

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*Summary.* A search for insect growth inhibitors in methanol extracts of soybean leaves resulted in isolation of pinitol. Pinitol caused a 50% reduction in weight gain ($ED_{50}$) of *Heliothis zea* larvae at about 0.7% when added to a synthetic diet. Although myo-inositol is a normal component of the insect diet, it also caused growth inhibition at higher concentrations; $ED_{50}$ 4%.

This paper reports work on soybean leaf (*Glycine max* [L] Merr) constituents as part of a systematic search for naturally occurring insect antifeeding or antigrowth substances in economically important crop plants[2]. Knowledge of these components would aid plant breeders in selecting for resistance to insect pests[3].

The bollworm (*Heliothis zea* [Boddie]) is an important pest on soybeans. The larvae eat the leaves or seed pods causing, in some cases, substantial plant damage[4]. Certain lines of soybeans, however, are relatively resistant to *H. zea*[5]. This paper reports a search for insect antifeeding or antigrowth substances in these resistant soybean lines using a feeding bioassay developed in this laboratory[6] as a guide in the isolation procedure. Test substances were mixed with *a*-cellulose and then incorporated in the wheat germ-casein diet. Newly hatched bollworm larvae were placed on diet pieces and were incubated at 26 °C for 12 days, whereupon the larvae were weighed and compared with a control group.

Extraction of freeze dried soybean leaves from the cultivar Davis or plant introduction PI 229358 with ethyl acetate, acetone or any solvent of lesser polarity gave, after removal of solvent, material which was inactive in an insect feeding bioassay using *H. zea* larvae on the artificial diet. On the other hand, extraction with acetone to remove inactive nonpolar substances followed by extraction with methanol and then water exhaustively in a Soxhlet extractor gave material in both latter extracts which cause a reduction in larval weight gain in the bioassay (table). This procedure was then used routinely to prepare active extracts. The results indicated that the active antigrowth material(s) was very polar and heat stable.

The methanol extracts were chromatographed on Sephadex LH-20 with methanol. Fractions were combined according to retention volumes and bioassayed. In this fashion, active material was concentrated and separated from much inactive material. The active fractions were then decolorized by treatment with Norite. The nearly colorless active syrupy residue showed no appreciable UV-absorption suggesting that the active material did not contain a UV-chromophore above 200 nm. This active fraction gave negative ninhydrin and Dragendorfs tests suggesting that it was free of nitro-

gen-containing substances. Filtration of the active material through strongly basic anion exchange resin, Dowex 1-4X, and bioassay of the eluted substances indicated that the active material(s) was not retained by the ion exchange resin, and hence must be a neutral substance. The methanol-soluble active eluents from the ion exchange resin were concentrated. Addition of acetone to these concentrates gave crystalline pinitol (3-0-methyl-chiro-inositol). The pinitol was identical by spectroscopic criteria (NMR, IR, MS) with authentic samples isolated from *Pinus ponderosa* and *Sequoia sempervirens* Endl. and the GLC retention times of the TMS ethers of all the samples were identical[7,8]. The yield of crystalline pinitol from both varieties of soybeans studied was about 1% of the dry weight. The isolated pinitol was active in the feeding bioassay (table).

Pinitol occurs frequently in legumes and may eventually be found in most members of this family[7-9]. Pinitol has previously been reported from soybeans[7,8] and is especially abundant in the leaves.[9].

Soybean resistance to *H. zea* feeding increases with increasing leaf maturity[10] which correlates well with increasing pinitol levels as the plant matures[11]. Pinitol mixed with inositol at equal or greater concentrations was fed to the *H. zea* larvae in the bioassay in order to test the possibility that pinitol may act as an antagonist to inositol present as a micronutrient in the diet. The weight gain of the larvae was still low and decreased with increasing levels of inositol so pinitol does not seem to be an inositol antagonist. Moreover, the addition of high levels of inositol to the diet (0.8–5.0%) results in severe inhibition of weight gain (table). In a simple choice test, *H. zea* larvae did not show any preference for the synthetic diet compared to that containing added pinitol. This indicates that pinitol does not act as a feeding repellant or attractant. Since soybean varieties which are resistant to *Heliothis* are also largely resistant to Mexican bean beetles[12] *(Epilachna varivestis* Mulsant), pinitol may also play a role in soybean resistance to this latter insect[13].

Antigrowth activity of soybean extracts and constituents

| Material added to diet | Percent of dried plant material | Percent added to synthetic diet in bioassay | Larval weight gain as percent of controls |
|---|---|---|---|
| Acetone extracts | 11 | 2.7 | 123 |
| | | 5.4 | 109 |
| Methanol extracts | 13 | 3.4 | 80 |
| | | 6.8 | 59 |
| Aqueous extracts | 9.5 | 2.3 | 82 |
| | | 4.6 | 50 |
| Pinitol | | 0.6 | 62 |
| | | 0.8 | 37 |
| | | 1.6 | 24 |
| | | 2.4 | 21 |
| | | 3.2 | 16 |
| Inositol | | 0.8 | 93 |
| | | 1.6 | 71 |
| | | 2.4 | 61 |
| | | 3.2 | 57 |
| | | 5.0 | 40 |

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## Phosphatase activity in testis and prostate of rats treated with embelin and *Vinca rosea* extract

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*Summary.* Daily administration of *Vinca rosea* Linn. extract orally and embelin s.c. to male albino rats caused significant rise in levels of acid and alkaline phosphatases of testis and prostate indicating altered metabolic function.

Vinblastine and vincristine isolated from *Vinca rosea* Linn. (Apocyanaceae) and embelin isolated from *Embelia ribes* Burm. (Myrsinaceae) have been reported to possess anticancerous and antifertility activity respectively in albino rats[2-4]. Vinblastine has been found to arrest mitosis at metaphase in rapidly proliferating cells[4]. I.p. administration of total alkaloids from *Vinca rosea* leaves produced degenerative changes in the spermatogenic elements of the testis of immature male rats[5]. Embelin (2,5-dihydroxy-3-undicyl-1,4-benzoquinone) has been reported to reduce the sperm motility in *Macaca bonnata*[6]. However, no effort has been made to study the influence of these compounds on bio-