structural elements (*2, 3, 10*). The data presently available indicate that benzoylphenylureas are not direct-acting chitin synthetase inhibitors, but rather that they are direct-acting serine protease inhibitors that block the conversion of chitin synthetase zymogen into active enzyme. Thus a variety of specific chymotrypsin inhibitors, which are not directed against other serine and nonserine active site proteases, are capable of selectively blocking insect chitin synthesis. These data provide evidence for the critical involvement of a chymotrypsin-like protease in insect chitin biosynthesis. Recently, Strauss *et al.* (*20*) implicated a chymotrypsin-like protease in the processing of the pre-segment of human secretory proteins. Furthermore, Green and Ryan (*21*) observed that injury to plant leaf surfaces elicits a hormonally mediated response resulting in the production of large quantities of polypeptide trypsin and chymotrypsin inhibitors. This plant defense system may have the same mode of action as the benzoylphenylureas.

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24. The chitin synthetase reaction mixture contained 0.025*M* tris, *p*H 8.0; 0.05*M* $MgCl_2$; 0.001*M* UDP-*N*-acetylglucosamine (UDP, uridine diphosphate); 0.05*M* *N*-acetylglucosamine; 0.075 μCi of [$^3$H]UDP-*N*-acetylglucosamine ([6-$^3$H]glucosamine, 6.6 Ci/mmole); 80 μg of pellet fraction protein; and $H_2O$ to make a total volume of 150 μl. The reaction mixture was incubated for 90 minutes at 28°C, and chitin synthesis was terminated by the addition of 5 percent trichloroacetic acid (4°C). The reaction mixture was filtered onto a glass fiber filter, washed with trichloroacetic acid and 95 percent ethanol, dried, and counted in a liquid scintillation spectrometer. The protein specificity of an inhibitory molecule was assessed by adding 500 μg of ovalbumin to a reaction mixture containing an amount of the test compound which would result in a 50 percent inhibition of enzyme activity. If the ovalbumin addition caused less than 10 percent deviation from the expected $I_{50}$ value, the compound was classified as a specific chitin synthetase inhibitor. The $I_{50}$ values were determined by graphical analysis of dose-response data; the standard error of these data is in the range of ± 10 percent. In substrate competition experiments, UDP-N-acetylglucosamine concentrations were 1 m*M* to 0.5 μ*M*. The specific activity of the $^3$H-labeled substrate was increased fivefold in these experiments. The reversible or irreversible nature of inhibition was determined by incubating (90 minutes at 28°C) 25 μl of pellet material in the presence of the test compound at a concentration in that volume known to produce 50 percent inhibition under usual assay conditions. This preliminary incubation was terminated, and the test compound was diluted sevenfold by the addition of the remainder of the assay components. The level of inhibition after a 90-minute assay was observed. A decrement greater than 10 percent from the expected $I_{50}$ value was interpreted as reversible inhibition.

25. We thank A. Glazer for helpful discussions, J. Stock and M. Delbrück for stimulating our interest in fungal biochemistry, and A. B. Borkovec for providing samples of dithiazolium iodide and Penfluron.

29 August 1980; revised 15 December 1980

## Disease Resistance: Incorporation into Sexually Incompatible Somatic Hybrids of the Genus *Nicotiana*

Abstract. *Somatic hybrid plants of* Nicotiana nesophila *and* N. stocktonii *with* N. tabacum *(cultivated tobacco) were produced by protoplast fusion. These combinations cannot be achieved with conventional sexual hybridization, yet are important in that the wild* Nicotiana *species are resistant to numerous diseases. Hybridity was verified by chromosome number, isoenzyme analysis, morphological characteristics, and genetic behavior. Local lesion-type resistance to tobacco mosaic virus has been observed in leaves of these somatic hybrid plants.*

Wild species of *Nicotiana* have been used to incorporate disease resistance into cultivated tobacco (*1*). The three *Nicotiana* species of the section *Repandae* (*N. nesophila*, *N. repanda*, and *N. stocktonii*) are among those species resistant to the most diseases of cultivated tobacco. Attempts to crossbreed these wild species with cultivated tobacco by conventional breeding techniques have been unsuccessful (*2, 3*). Two of these species, *N. nesophila* and *N. stocktonii*, have been crossed with *N. tabacum* (*4*) by means of ovule culture in vitro. How-

Table 1. Comparison of morphological characteristics of *N. tabacum* + *N. nesophila* (NN + Su/Su) somatic hybrid plants with the two parental species.

| Plant | Flower | | Leaf (cm) | | Pollen viability (%) |
|---|---|---|---|---|---|
| | Color | Length* (cm) | Length of blade* | Maximum width* | |
| *N. tabacum* (Su/su) | Dark pink | 5.28 ± 0.07 | 23.00 ± 2.48 | 7.25 ± 1.23 | 97.5 |
| NN + Su/Su (somatic hybrids) | Light pink | 5.09 ± 0.08 | 18.25 ± 1.31 | 8.13 ± 0.32 | 55.3 |
| *N. nesophila* (NN) | White | 4.95 ± 0.07 | 13.14 ± 0.46 | 9.29 ± 0.48 | 96.5 |

*Measurement expressed as mean ± standard error with differences significant, with $P$ less than .05, for each group of plants.

Table 2. Segregation of the Su locus controlling leaf pigmentation in sexual progeny of *N. tabacum* + *N. nesophila* (NN + Su/Su) somatic hybrid plants.

| Sexual cross | Dark green | Light green | Albino |
|---|---|---|---|
| (NN + Su/Su) × *N. tabacum* (su/su) | 58 | 36 | 0 |
| (NN + Su/Su) × *N. nesophila* | 11 | 14 | 0 |
| *N. tabacum* (su/su) × (NN + Su/Su) | 3 | 2 | 0 |
| (NN + Su/Su) × self | 11 | 35 | 1 |


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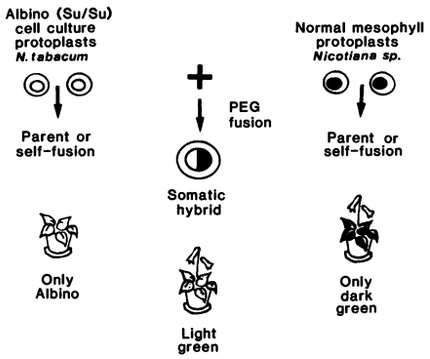


Fig. 1 (left). Selection scheme used for the recovery of somatic hybrid plants after protoplast fusion of *N. tabacum* (Su/Su) cell culture protoplasts with leaf mesophyll protoplasts of wild *Nicotiana* species. Fig. 2 (right). (A) Leaves of *N. nesophila*, NN + Su/Su somatic hybrid, and *N. tabacum* (left to right). Note the presence of single spots on the leaf surface of NN + Su/Su. (B) Somatic root tip chromosomes of NSt + Su/Su somatic hybrid plant, with $2n = 8x = 96$ chromosomes. This represents the expected amphiploid chromosome number. (C) Esterase isoenzymes separated by polyacrylamide gel electrophoresis. Isoenzymes of *N. tabacum*, NSt + Su/Su somatic hybrids, *N. stocktonii*, and a mixture of the *N. stocktonii* and *N. tabacum* extracts (top to bottom). Note the appearance of the band that appears only in the somatic hybrid and is absent from the mixture among the major bands (arrow). Unique band is intermediate to the parental bands in mobility.

ever, *N. repanda*, the species resistant to more diseases of cultivated tobacco than any other *Nicotiana* species (*5*), cannot be crossed with *N. tabacum* even by means of ovule culture (*4*). We have attempted to utilize somatic hybridization to combine the *Repandae* species with *N. tabacum*.

Leaf mesophyll protoplasts from each *Repandae* species and protoplasts from cell suspension cultures of Su/Su albino *N. tabacum* were isolated as described (*6, 7*). As the albino mutation is semidominant, somatic hybrids should appear light green in color (Fig. 1). Protoplasts of *Repandae* species and Su/Su *N. tabacum* were mixed in 1:1 ratio and agglutinated with PEG (*8*). The PEG was eluted with the use of a solution having both a high concentration of $Ca^{2+}$ ions and high *p*H (*8*), so that the final heterokaryotic fusion frequency was approximately 10 percent in each experiment. After fusion, the protoplasts were cultured in stationary liquid medium 8p (*9*). After 1 month the resulting friable callus was resuspended and plated onto solid MS medium with 5 $\mu M$ 6-benzyladenine for shoot regeneration (*8*). Light green shoots, intermediate in pigmentation between the two parental lines, were separated and transferred to rooting medium (*8*). Light green plants were recovered from fusion experiments of *N. nesophila* (NN + Su/Su) and *N. stocktonii* (NSt + Su/Su) with *N. tabacum* (Fig. 2A), but not with *N. repanda*.

Numerous criteria were used to verify the hybrid nature of the light green plants. The chromosome number could not be used to distinguish the NN + Su/Su and NSt + Su/Su plants from polyploid parental plants resulting from homokaryotic fusion products. The number of metacentric chromosomes and of nucleoli was also identical in *N. tabacum*, *N. nesophila*, and *N. stocktonii*. Nonetheless, all 10 NN + Su/Su and 11 NSt + Su/Su plants counted contained $2n = 8x = 96$ chromosomes (Fig. 2B). This chromosome stability is of interest because most somatic hybrid plants have aneuploid chromosome number (*10*). Most morphological characteristics of the somatic hybrids were intermediate, as expected. Characters examined included leaf shape, leaf size, plant height, floral color, and floral dimensions (Table 1). Isoenzymes of both esterase and aspartate aminotransferase were examined. Whereas *N. nesophila* and *N. stocktonii* have two esterase isoenzymes, *N. tabacum* has only one isoenzyme. The three parental isoenzymes and a band found only in the hybrid were present in both NN + Su/Su and NSt + Su/Su plants (Fig. 2C). All parental aspartate aminotransferase isoenzymes and a band present only in the NN + Su/Su and NSt + Su/Su plants have been observed. These results for the aminotransferase are consistent with isoenzyme analysis of Ng + Su/Su somatic hybrids reported earlier (*8*).

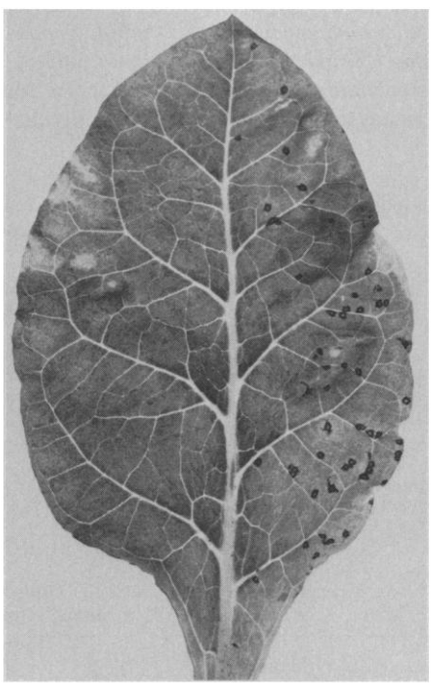

Fig. 3. The right half of this detached leaf of NN + Su/Su was inoculated with TMV. Local lesions, representing a hypersensitive response, formed on this half of the leaf. Virus did not spread to the left half of the leaf.

In *N. tabacum*, tobacco mosaic virus (TMV) replicates and invades a plant systemically, resulting in mosaic disease (*11*). In *N. nesophila*, inoculation with TMV results in production of local lesions. These lesions reflect a hypersensitive response that results in localization of the virus infection (*12*). Disease resistance to TMV was tested with detached leaves. When the right half of detached leaves of *N. nesophila* and NN + Su/Su plants were inoculated with TMV, local lesions were produced (Fig. 3). Inoculated *N. tabacum* leaves did not produce local lesions. After 72 hours, both the inoculated and uninoculated halves of all leaves were collected and tested for TMV, with the use of leaves of *N. glutinosa* as virus indicator. Virus had spread to the uninoculated half of the leaf in *N. tabacum*, while TMV was restricted to the inoculated half of the leaf in *N. nesophila* and NN + Su/Su. Local lesion-type resistance has resulted in field resistance in tobacco (*13*). This detached leaf test for resistance has been verified in intact NN + Su/Su plants, and TMV resistance has been observed in four separate NN + Su/Su clones. This represents the first instance of incorporation of disease resistance into sexually incompatible somatic hybrids.

The NN + Su/Su plants have flowered and have approximately 50 percent pollen viability (Table 1) with reduced

fertility. Nonetheless, these hybrids have been successfully backcrossed to the parental *Nicotiana* species, particularly with NN + Su/Su plants as female parents. Only one clone of NN + Su/Su could be self-fertilized. Although seed viability was low, germinating seed has been recovered in all cases and has segregated for the Su leaf pigmentation character (Table 2). The data obtained thus far are consistent with segregation ratios expected in amphiploid interspecific hybrids of *Nicotiana* (*14*). The Su locus of *N. tabacum* is associated with the formation of single and double spots on light green Su/su *N. tabacum* plants (*15*). The appearance of double spots (dark green area adjacent to albino) on the light green surface of both NN + Su/Su and NSt + Su/Su plants implies genetic recombination between the wild species and the *N. tabacum* genome. Such recombination may facilitate introgression of traits such as disease resistance into cultivated tobacco. In addition, double spots have been observed on all light green plants of NN + Su/Su backcrossed to *N. nesophila* or to *N. tabacum*, but as in *N. tabacum*, have not been observed on dark green plants. Consequently, the constancy of genetic behavior of the Su locus and segregation of the Su gene in backcross and self-fertilized progeny of NN + Su/Su provide additional verification that the protoplast-derived light green plants are somatic hybrids.

No NR + Su/Su somatic hybrids have been recovered. This is consistent with the results of ovule culture techniques (*4*). The NR + Su/Su cell hybrids were observed to undergo mitosis, but no light green shoots were isolated. The NN + Su/Su and NSt + Su/Su somatic hybrids will be screened for resistance to other diseases. Production of somatic hybrids may complement other novel methods utilizing plant protoplasts to incorporate genetic variability into crop species (*16*).

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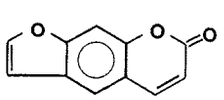
17. We thank Joan Markiewicz for preparation of Fig. 1 and Drs. S. M. Reed and W. R. Sharp for helpful comments. Supported by USDA grant 7900065 and a SUNY UAC grant to D.A.E.

15 September 1980; revised 18 March 1981

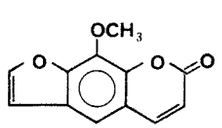
## Natural Toxicants in Human Foods: Psoralens in Raw and Cooked Parsnip Root

Abstract. *Parsnip root contains three photoactive, mutagenic, and photocarcinogenic psoralens in a total concentration of about 40 parts per million. These chemicals are not destroyed by normal cooking procedures (boiling or microwave); thus humans are exposed to appreciable levels of psoralens through the consumption of parsnip and possibly other psoralen-containing foodstuffs. The toxicologic consequences to man of such exposure may be speculated on the basis of medicinal and laboratory studies, but epidemiologic data are not available.*

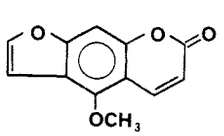
Man is exposed to thousands of natural and synthetic chemicals from a great number of environmental and nutritional sources. Many of these chemicals are necessary to sustain life itself, while others are harmless considering the levels encountered, their inherent toxicological properties, and the usually efficient array of detoxication and excretory mechanisms that act upon them in man. Still other chemicals represent significant toxicological hazards, and their interaction with man may result in clear and readily definable dangers such as the possibility of acute poisonings, effects on reproduction or other body functions, and genetic effects (mutations). The potential significance to man of naturally occurring toxicants in foods may be equal to or greater than that of man-made chemicals and must not be overlooked. We report here that roots of parsnip, *Pastinaca sativa*, a vegetable available in most supermarkets, contain appreciable levels of three phototoxic, mutagenic, and photocarcinogenic linear furocoumarins (psoralens) and that these chemicals are not destroyed by normal cooking procedures. Thus, humans are exposed to psoralens in the diet, with so far undefined toxicological consequences.



Psoralen



Xanthotoxin



Bergapten

Fig. 1. Linear furocoumarins (psoralens) from parsnip root.

California-grown parsnip roots were obtained from a local supermarket, they were washed thoroughly in tap water, and the ends and crowns were cut off. Each root was quartered lengthwise, then cut crosswise into approximately 1-cm pieces. Samples (100 g each) were cooked by boiling in water or by microwave radiation until tender (*1*). The cooked samples, as well as samples of uncooked parsnip root, were homogenized in water and extracted five times with ethyl acetate. Portions of the extracts equivalent to 5 g of parsnips were then analyzed by thin-layer chromatography (TLC) (*2*) for resolution of the psoralens present. Gel areas on the developed plates corresponding to authentic psoralen, xanthotoxin (8-methoxypsoralen), and bergapten (5-methoxypsoralen) (Fig. 1), as visualized under long-wavelength ultraviolet light, were eluted with diethyl ether and then subjected to gas-liquid chromatography (GLC) for quantitative measurements (*3*). The identities of the three psoralens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