Influences of Stock and Scion on Alkaloid Synthesis and Transformation

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It is desirable and necessary to reexamine the influences of stock and scion on alkaloid synthesis and transformation using plants which behave differently with respect to alkaloid formation (4). Experiments were conducted in the greenhouse for 3 years in an attempt to separate the effects of root, or stock, from those of the top, or scion, on alkaloid synthesis and transformation. Some of the grafting combinations reported in this paper resembled those prepared previously by Dawson (2), but plants with various degrees of ability in alkaloid synthesis were included in this study.

Materials and Methods

The following plants were used in these experiments: Lycopersicon esculentum Mill cv. Rutgers (Tom); Nicotiana tabacum L. cv. Maryland Robinson Medium Broadleaf tobacco, a strain homozygous for normal nicotine production (Md); N. tabacum cv. Swarr-Hibshman Pennsylvania cigar tobacco (SH); N. tabacum L., cv. Baur, a low nicotine tobacco; N. glauca Grah. (Gla); and N. glutinosa L. (Glat). The plants were first grown in 10-cm pots. When they were about 10 cm tall, 2 plants to be grafted were set close together in 10-liter pails. Ten pails, each containing 2 plants of 10 graft combinations, plus 3 pails each containing 1 plant of the 6 species or varieties alone, a total of 118 pails, were prepared.

The grafts were made by approach grafting. Clothespins were used to hold the stems of the 2 plants together. Two weeks later, the top of 1 plant and the root of the other were severed. It was found necessary to place the grafted plants under a cloth shade at high moisture levels for several days after cutting as the conductive system through the graft was unable to carry the load of normal transpiration. The nitrogen supply of the plants was kept at a minimum until the original roots of the scion were removed and then raised to encourage alkaloid synthesis. Some of the excised tops were analyzed to provide an estimate of the alkaloid already present in the tops of alkaloid synthesizing species when they were cut loose from their own roots and left on a stock of a practically nonalkaloid-synthesizing plant like tomato.

The plants were not decapitated, and were harvested after 9 to 11 weeks. Only the tops, including leaf and stalk, were analyzed immediately after harvesting. The stalk and leaf were separated and the floral parts and small suckers were discarded. An effort was made to take representative portions of both leaf and stalk. Samples of the leaf material of the tomato and N. glutinosa were cut with shears into several piles in a way to insure that all piles contained equal proportions of basal to apical tissue and immature to overmature tissue. One pile was then taken for analysis of green tissue and another for moisture determination.

A narrow portion cut transversely across the middle of every leaf sample of N. tabacum varieties gave a subsample reasonably representative of the whole. Subsamples of the stalk for analysis and for moisture determination were taken from various portions of the stalks.

All the analytical subsamples were macerated in a blender with acetone adjusted to 50% in the final solution, taking into consideration the water in the tissue (5). This acetone macerate was then filtered with suction, and one portion was used for paper chromatography to determine the amount of nicotine, nor nicot ine, and anabasine (5, 12), and another to determine total quantity of alkaloids by steam distillation and ultraviolet spectrophotometry (15).

Results on alkaloid composition after leaf curing are also available, but are omitted from this report because little changes were observed in most cases except in Maryland Robinson in which most of the nicotine was converted to nor nicot ine during curing (13).

Results and Discussion

Nor nicot ine-nicotine are readily interconvertible (14) through transmethylation and oxidative demethylation (10). Nor nicot ine is formed before nicotine in the root as well as the shoot of Nicotiana plants (11, 13). In nicotine biosynthesis, nor nicot ine can serve as an immediate precursor of nicotine (6, 11). The conversion of nicotine to nor nicot ine occurred only at a later stage of plant development (2, 13).

The graft combinations used, years the special graft survived, total number of plants harvested, and the average dry weight of leaf and stalk obtained plus their alkaloid content and composition are shown in table I. Certain combinations, especially tomato-on-N. glutinosa, did not survive well. Five or more of the original 10 grafted plants of the others were still
in good condition at the end of the experiment. Three scions succeeded in establishing adventitious roots in the soil and were discarded. There was a great deal of difference in the difficulty of making the various grafts depending on the growth habit of the 2 plants involved.

In intact plants, Rutgers tomato plants produced no compounds recognized as alkaloids by the methods used (table I). The select strain of Maryland Robinson Medium Broadleaf tobacco used contains nicotine predominantly with a small amount of nornicotine in the green plant. Most of this nicotine is transformed to nornicotine during air curing. Swarr-Hibshman Pennsylvania Seedleaf tobacco contains considerable quantities of nicotine. Baur tobacco relatively small quantities of nicotine. N. glutinosa contains predominately nornicotinie, but with some nicotine, and N. glauca contains predominantly anabasine.

The tops of plants composed of tomato scions on Maryland Robinson stock contain nicotine predominately with small amounts of nornicotine. The tops of plants composed of tomato scions on N. glutinosa stocks contain moderate amounts of nicotine, but only minute amounts of nornicotine. It appears that in the tomato shoot, the alkaloid conversion system is in favor of nornicotine to nicotine rather than nicotine to nornicotine.

The tops of plants of the Maryland Robinson tobacco scions, Swarr-Hibshman tobacco scions, and N. glutinosa scions, all on tomato stock, contain appreciable quantities of nicotine. These results confirmed the previous experiments with N\textsuperscript{15} (13) that considerable amounts of alkaloid, including nicotine and nornicotine, were formed in the shoot. Solt (9) also provided qualitative evidence on the nicotine synthesis in the shoot. In the present 3 cases, the amount of alkaloid found in the grafted shoots on tomato stock was 4\%, 5\%, and 33\% respectively of the total alkaloid found in the intact Maryland Robinson, Swarr-Hibshman, and N. glutinosa plants. These results indicated a much higher alkaloid synthesis in the shoot than that estimated by Dawson and Solt (3) for nontopped plants. Early work by Mashkovtsev and Sirotenko (7) reported 0.04\% of the total nicotine to be produced in the "aerial part" of the plant. Mothes and Romeike (8) indicated a synthesis of alkaloids occurred in fruit and seeds, yet neither the quantity nor the quality reached the normal detectable level when these organs were ripened artificially by separation from the plant or by development on foreign roots.

The tops of plants composed of Swarr-Hibshman scions on Baur stocks contained an amount of nicotine comparable to that of intact Baur plants, while the tops of the reciprocal grafts contained large amounts of nicotine. The tops of plants composed of N. glutinosa scions on Baur tobacco stocks contained...
very little alkaloid, while the tops of plants composed of Baur scions on *N. glutinosa* stocks contained considerable amounts of alkaloid considering the small size of the plants. These results suggest that the low alkaloid content of Baur tobacco results from a deficiency in the alkaloid-synthesis mechanism in the roots.

The tops of plants composed of Maryland Robinson scions on *N. glauca* roots contained relatively little alkaloid, and a majority of this was anabasine, but both nicotine and nornicotine were present. The tops of plants composed of *N. glauca* scions on Maryland Robinson stocks contained much larger quantities of nornicotine and especially anabasine than the reciprocal graft. This would be expected as it combines the root-synthesis mechanism found in Robinson with the leaf synthetic mechanism for anabasine found in *N. glauca* (1). The high content of nor-nicotine indicated that nornicotine was formed directly in the root of Maryland Robinson (13) and was translocated to the *N. glauca* scion.

The tops of plants composed of Swarr-Hibshman scions on *N. glauca* stocks contain small amounts of anabasine and nicotine and traces of nornicotine, while the tops of reciprocal grafts contain no nicotine and considerably larger quantities of anabasine. Previous hypotheses explain the larger amounts of anabasine as being synthesized in the *N. glauca* leaves (1), but they do not explain the absence of nicotine.

The tops of plants composed of Baur tobacco scions and *N. glauca* stocks contained predominantly anabasine with a small amount of nicotine and nornicotine, while the tops of reciprocal grafts contained a similarly large amount of anabasine but also no nicotine.

The tops of plants composed of *N. glutinosa* scions and *N. glauca* stocks contained small amounts of anabasine and nornicotine and very small amounts of nicotine, while in the tops of the reciprocal grafts the amounts of anabasine and nornicotine were generally similar, but again no nicotine was found.

All the grafted plants with *N. glauca* scion had no nicotine regardless of whether the grafted stock was nicotine type or nornicotine type. Three possible explanations are suggested as follows: A) The formation of anabasine in the *N. glauca* scion is at the expense of nicotine, although it had been demonstrated that anabasine was not formed by rearrangement of the nicotine molecule (14); B) there is an active demethylation system in *N. glauca* scion in which nicotine was converted to nornicotine; and C) the pathway of nicotine formation was interrupted at the stage of nornicotine because of the absence of the specified methylation system in *N. glauca* scion. Additional investigations are underway to verify these hypotheses.

Nornicotine formation, in Maryland Robinson or *N. glutinosa*, appeared to need both scion and stock of the nornicotine-dominant plant to produce a normal amount of that alkaloid. The summation of the average amount of nornicotine found in grafted plants with a Maryland Robinson scion and those grafted with Maryland Robinson stocks or a similar summation of grafted plants with *N. glutinosa* scions and *N. glutinosa* stocks is much lower in comparison with the respective intact ones. The average nornicotine content in all other grafted plants from table I, which contains no parts from Maryland Robinson or *N. glutinosa*, is only 0.01 g/plant.

Anabasine formation in the root or shoot of *N. glauca*, however, was not affected by the grafting of a foreign scion or stock. The summation of average anabasine content in grafted plants composed of *N. glauca* scions and that of *N. glutinosa* stocks is 0.62 to 0.41 in comparison with 0.67 and 0.46 in green and cured samples respectively, while the anabasine content in all other grafted plants from table I, which contains no parts from *N. glauca*, is only 0.001 to 0.003 respectively.

The tomato shoot or root when used as a scion or stock caused a decrease but not an absence of alkaloid in the grafted plants.

**Summary**

Experiments were conducted to study the influence of stock and scion in alkaloid synthesis and transformation. Reciprocal grafts of different combinations of plants were made including *Lycopersicum esculentum* Mill cv. Rutgers; *Nicotiana tabacum* L. cv. Maryland Robinson Medium Broadleaf tobacco, a selected nornicotine strain; *N. tabacum* cv. Swarr-Hibshman Pennsylvania cigar tobacco; *N. tabacum* L. cv. Baur, a low nicotine tobacco; *N. glauca* Grah.: and *N. glutinosa* L. The top of *Nicotiana* plants grafted onto tomato stock produced an appreciable amount of alkaloids, indicating the capability of alkaloid synthesis in a scion in the absence of its own roots. Nicotine was absent in grafted plants composed of tops of *N. glauca* as scion, but nornicotine was always present. Nornicotine-dominant plants, *N. glutinosa* and Maryland Robinson tobacco, were dependent on both root and shoot to produce a normal level of nornicotine. The summation of alkaloids found in the shoot and the root grafted to a foreign stock or scion was always lower than the amount of alkaloid found in the respective intact plants. On the other hand, anabasine-dominant plants, *N. glauca*, appeared not to be dependent on a combination of root and shoot to produce a normal level of anabasine. The summation of alkaloids found in shoot and root grafted to other plants was about the same as that found in intact *N. glauca* plants.

**Acknowledgment**

W. H. Eoff, formerly with the Field Crops Research Branch, Agricultural Research Service, conducted the analytical work on alkaloids.

**Literature Cited**

Alteration of Oxidative Enzymes in Potato Tuber Tissue by Infection with Phytophthora infestans 1, 2

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Knowledge has accumulated as to the role of pectinase in plant disease (1, 12, 13, 14). In this paper evidence will be presented that pectinase preparations can cause a respiratory acceleration and increase of the activities of polyphenol oxidase and peroxidase in host tissues. Uritani and Stahmann (11) showed that the peroxidase activities which increased in the diseased sweet potato tuber tissues can be separated into several different components by means of starch gel electrophoresis. Therefore, it may be reasonable to suppose that a solution to the problem of oxidative enzymes in diseased tissues will not be reached, unless the physiological meaning of the different components of peroxidase is made clear. In the present study the proteins extracted from the tissue neighboring the infected cells were fractionated by chromatographic and electrophoretic methods, and assayed for peroxidase and polyphenol-oxidase activities after infection by a compatible and an incompatible strain of Phytophthora infestans. The compatible strain, race 1, can successfully infect the host tissue. The incompatible strain, race 0, can invade the potato cells as well as race 1, but because the cells of the variety Kennebec has the R, gene for resistance, a marked browning occurs soon after inoculation and the infection is not successful.

Methods and Materials

Preparation of Slices. The potato variety Kennebec was used in most of the experiments. In order to obtain heavy infection of the cut surface of potato tubers, suspensions of zoospores which germinated from zoosporangia were concentrated by centrifugation at 1000 rpm for 3 minutes and inoculated onto freshly cut surfaces of potato tubers. The tubers were cut in half; one half was inoculated or treated and then both halves were kept in a dark moist cham-

1 Revised manuscript received Sept. 16, 1963.
2 Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a research grant from the Herman Frasch Foundation.