

# THE ACCUMULATION AND MOVEMENT OF NICOTINE IN RECIPROCAL GRAFTS BETWEEN TOBACCO AND TOMATO PLANTS

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## Introduction

IN 1934-35, and subsequent crop-years, a large number of grafts was made at the Imperial Agricultural Research Institute in connection with studies on the leaf-curl diseases of tobacco and reported upon by Pal and Tandon.<sup>1</sup> As the grafts included some between tobacco and plants of other species, it was considered a suitable opportunity to study the problem of the accumulation and movement of nicotine. Grafe and Linsbauer<sup>2</sup> had already shown that *Nicotiana affinis* which does not normally possess nicotine in any of its parts, has as much as 1.67 per cent. when grafted on *N. Tabacum*. But there was obviously a need for further work on this problem.

## Materials and Methods

The tobacco variety used in these experiments was *N. rustica* Type 18, which has a relatively high nicotine content. The Pritchard variety of tomato was selected as it grows well in Northern India. In the earlier experiments, ordinary cleft grafting was done, the grafts being made reciprocally, *i.e.*, some with tobacco as stock and tomato as scion, and some the other way about. The scions in each case showed wilting in the daytime for about three days but after that regained normal turgidity; new growth was visible after about a week. In the later experiments (1937-38) lateral grafts were made. In 1934-35, the experiment was of a preliminary nature.

In the following crop-year the grafting experiments were repeated on an extended scale, using the same varieties and technique as previously. The tobacco seed was sown and the seedlings transplanted on the 31st August and 2nd October respectively. The grafts were made on 30th November—4th December. Two grafts with tomato scions and tobacco stocks and two of the reciprocals were pulled up for analysis on the 9th December, *i.e.*, ten days after grafting. Thereafter four grafts (two of each reciprocal)

were pulled up every fortnight and analysed. Two non-grafted plants each of tobacco and tomato were pulled up for analysis at the same time as the first batch of grafts.

Nicotine was estimated by the method developed by Worsley<sup>3</sup> using freshly prepared lacmoid solution as the indicator. The method is quicker and simpler and gives values agreeing with those of Young's modification of Keller's method.<sup>4</sup>

### *Experimental*

#### *1. Nicotine Content in Non-grafted Plants of Tobacco and Tomato*

As mentioned in the preceding section, two plants each of tobacco and tomato were analysed for nicotine content at the same time as the first batch of grafts in 1935-36. The percentage nicotine based on air-dry samples was as follows:—

|                      | Tobacco     |             | Tomato          |
|----------------------|-------------|-------------|-----------------|
|                      | Plant No. 1 | Plant No. 2 |                 |
| Roots .. .. .        | 0.45        | 0.24        | Nil (or traces) |
| Lower stem .. .. .   | 0.53        | 0.38        | "               |
| Upper „ .. .. .      | 0.57        | 0.16        | "               |
| Lower leaves .. .. . | 1.26        | 0.97        | "               |
| Upper „ .. .. .      | 0.85        | 0.77        | "               |
| Fruits .. .. .       | ..          | ..          | "               |

It will be observed that while the alkaloid is present in all parts of the tobacco plants, it is entirely wanting in the tomato plants. In the former it is present to the largest extent in the leaves, the stem and roots containing considerably smaller amounts. There is a variation in the nicotine content of the two tobacco plants, one of them, possessing less nicotine than the other in all the plant parts studied.

#### *2. Nicotine Content in Grafted Plants of Tobacco and Tomato*

(1) 1934-35.—A preliminary experiment was carried out in this year and the results are summarised below:—

##### *Nicotine percentage on air-dry material*

|                       |      |
|-----------------------|------|
| Tobacco stock .. .. . | 9.37 |
| Tomato scion .. .. .  | 1.27 |
| Tomato stock .. .. .  | Nil  |
| Tobacco scion .. .. . | 0.35 |

(2) 1935-36.—The results are given in detail in Tables I and II.

TABLE I

*Percentage of Nicotine Content (on air-dry samples) of Grafts  
where Tomato was Scion and Tobacco was Stock*

| Date of sampling |       |    |    | Roots  | Stem |       | Leaves |       | Fruit |       |
|------------------|-------|----|----|--------|------|-------|--------|-------|-------|-------|
|                  |       |    |    |        | Old  | Young | Old    | Young | Green | Ripe  |
| Tomato Scion     |       |    |    |        |      |       |        |       |       |       |
| 9-12-35          | Pl. 1 | .. | .. | Nil    | ..   | ..    | ..     | ..    | ..    | ..    |
|                  | " 2   | .. | .. | "      | ..   | ..    | ..     | ..    | ..    | ..    |
| 24-12-35         | " 1   | .. | .. | "      | ..   | ..    | 0.87   | ..    | ..    | ..    |
|                  | " 2   | .. | .. | "      | ..   | ..    | 1.19   | ..    | ..    | ..    |
| 8- 1-36          | " 1   | .. | .. | "      | ..   | ..    | 0.35   | ..    | ..    | ..    |
|                  | " 2   | .. | .. | 0.30   | ..   | 2.99  | 1.52   | ..    | ..    | ..    |
| 22- 1-36         | " 1   | .. | .. | 0.07   | ..   | ..    | 2.62   | ..    | ..    | ..    |
|                  | " 2   | .. | .. | Nil    | ..   | ..    | 1.59   | ..    | ..    | ..    |
| 5- 2-36          | " 1   | .. | .. | 0.31   | ..   | 2.10  | 1.50   | ..    | ..    | ..    |
|                  | " 2   | .. | .. | 0.44   | ..   | 2.56  | 1.37   | ..    | ..    | ..    |
| 19- 2-36         | " 1   | .. | .. | 0.15   | 0.04 | 1.98  | 1.22   | ..    | ..    | ..    |
|                  | " 2   | .. | .. | 0.03   | Nil  | 1.30  | 1.37   | ..    | ..    | ..    |
| 4- 3-36          | " 1   | .. | .. | Nil    | ..   | 0.81  | 0.48   | ..    | ..    | ..    |
|                  | " 2   | .. | .. | 0.04   | ..   | 1.46  | 0.71   | ..    | ..    | ..    |
| 19- 3-36         | " 1   | .. | .. | 0.04   | ..   | 1.22  | 0.85   | ..    | ..    | ..    |
|                  | " 2   | .. | .. | N. T.* | ..   | 1.42  | 0.85   | Nil   | ..    | ..    |
| 11- 4-36         | " 1   | .. | .. | 0.01   | ..   | 0.77  | 0.72   | "     | ..    | Nil   |
|                  | " 2   | .. | .. | 0.05   | ..   | 1.18  | 1.19   | N. T. | ..    | N. T. |
| Tobacco Stock    |       |    |    |        |      |       |        |       |       |       |
| 9-12-35          | Pl.1  | .. | .. | 0.81   | 1.65 | ..    | 4.50   | ..    | ..    | ..    |
|                  | " 2   | .. | .. | 0.97   | 1.70 | ..    | 4.94   | ..    | ..    | ..    |
| 24-12-35         | " 1   | .. | .. | 1.13   | 1.86 | ..    | 6.76   | ..    | ..    | ..    |
|                  | " 2   | .. | .. | 1.13   | 2.43 | ..    | 6.32   | ..    | ..    | ..    |
| 8- 1-36          | " 1   | .. | .. | 1.22   | 1.66 | ..    | 8.59   | ..    | ..    | ..    |
|                  | " 2   | .. | .. | 1.18   | 1.58 | ..    | 7.94   | ..    | ..    | ..    |
| 22- 1-36         | " 1   | .. | .. | 0.77   | 1.18 | 1.26  | 8.02   | 3.16  | ..    | ..    |
|                  | " 2   | .. | .. | 0.85   | 1.26 | 1.05  | 8.42   | 2.19  | ..    | ..    |
| 5- 2-36          | " 1   | .. | .. | 0.89   | 1.74 | 1.56  | 7.29   | 2.11  | ..    | ..    |
|                  | " 2   | .. | .. | 0.61   | 1.50 | 1.05  | 5.83   | 2.59  | ..    | ..    |
| 19- 2-36         | " 1   | .. | .. | 0.81   | 1.46 | ..    | 9.15   | ..    | ..    | ..    |
|                  | " 2   | .. | .. | 0.65   | 0.69 | 1.38  | 1.86   | 1.86  | ..    | ..    |
| 4- 3-36          | " 1   | .. | .. | 0.65   | 0.73 | ..    | ..     | 1.83  | ..    | ..    |
|                  | " 2   | .. | .. | 0.60   | 1.01 | ..    | ..     | 1.13  | ..    | ..    |
| 19- 3-36         | " 1   | .. | .. | 0.49   | 0.49 | ..    | 3.20   | 1.50  | 0.28  | 0.57  |
|                  | " 2   | .. | .. | 0.59   | 0.93 | ..    | 0.86   | 1.49  | ..    | ..    |
| 11- 4-36         | " 1   | .. | .. | 0.45   | 0.31 | ..    | ..     | ..    | ..    | ..    |
|                  | " 2   | .. | .. | 0.53   | 0.57 | ..    | 3.08   | 2.87  | ..    | 0.61  |

\* N. T. = nil or traces.

Tables I and II demonstrate that whereas in the grafts where the tobacco is used as the stock and the tomato as the scion, the latter distinctly shows the presence of nicotine, in the reciprocal grafts the tomato stock is devoid

TABLE II

*Percentage of Nicotine Content (on air-dry samples) of Grafts  
where Tobacco was Scion and Tomato was Stock*

| Date of sampling     |       |    |    | Roots | Stem  |       | Leaves |        | Fruit |       |
|----------------------|-------|----|----|-------|-------|-------|--------|--------|-------|-------|
|                      |       |    |    |       | Old   | Young | Old    | Young  | Green | Ripe  |
| <i>Tobacco Scion</i> |       |    |    |       |       |       |        |        |       |       |
| 9-12-35              | Pl. 1 | .. | .. |       | Nil   | ..    | ..     | ..     | ..    | ..    |
|                      | 2     | .. | .. |       | ..    | ..    | ..     | ..     | ..    | ..    |
| 24-12-35             | 1     | .. | .. |       | ..    | ..    | ..     | Nil    | ..    | ..    |
|                      | 2     | .. | .. |       | ..    | ..    | ..     | 0.12   | ..    | ..    |
| 8- 1-36              | 1     | .. | .. |       | ..    | ..    | ..     | N. T.* | ..    | ..    |
|                      | 2     | .. | .. |       | ..    | ..    | Nil    | Nil    | ..    | ..    |
| 22- 1-36             | 1     | .. | .. |       | ..    | ..    | ..     | ..     | ..    | ..    |
|                      | 2     | .. | .. |       | ..    | ..    | ..     | 0.07   | ..    | ..    |
| 5- 2-36              | 1     | .. | .. |       | ..    | ..    | N. T.  | Nil    | ..    | ..    |
|                      | 2     | .. | .. |       | ..    | ..    | ..     | N. T.  | ..    | ..    |
| 19- 2-36             | 1     | .. | .. |       | 0.06  | 0.06  | ..     | 0.02   | ..    | ..    |
|                      | 2     | .. | .. |       | 0.04  | Nil   | ..     | Nil    | Nil   | ..    |
| 4- 3-36              | 1     | .. | .. |       | Nil   | ..    | ..     | ..     | N. T. | ..    |
|                      | 2     | .. | .. |       | ..    | ..    | ..     | 0.03   | Nil   | ..    |
| 19- 3-36             | 1     | .. | .. |       | N. T. | ..    | N. T.  | 0.01   | ..    | 0.02  |
|                      | 2     | .. | .. |       | Nil   | ..    | 0.06   | 0.01   | 0.01  | Nil   |
| 11- 4-36             | 1     | .. | .. |       | 0.02  | ..    | 0.03   | ..     | ..    | N. T. |
|                      | 2     | .. | .. |       | 0.04  | ..    | Nil    | ..     | ..    | Nil   |
| <i>Tomato Stock</i>  |       |    |    |       |       |       |        |        |       |       |
| 9-12-35              | Pl. 1 | .. | .. | Nil   | Nil   | ..    | Nil    | ..     | ..    | ..    |
|                      | 2     | .. | .. | ..    | ..    | ..    | N. T.  | ..     | ..    | ..    |
| 24-12-35             | 1     | .. | .. | ..    | ..    | ..    | Nil    | ..     | ..    | ..    |
|                      | 2     | .. | .. | ..    | ..    | ..    | ..     | ..     | ..    | ..    |
| 8- 1-36              | 1     | .. | .. | ..    | ..    | ..    | N. T.  | ..     | ..    | ..    |
|                      | 2     | .. | .. | ..    | ..    | ..    | Nil    | ..     | ..    | ..    |
| 22- 1-36             | 1     | .. | .. | ..    | ..    | ..    | ..     | ..     | ..    | ..    |
|                      | 2     | .. | .. | N. T. | ..    | Nil   | ..     | Nil    | ..    | ..    |
| 5- 2-36              | 1     | .. | .. | Nil   | 0.01  | ..    | ..     | ..     | ..    | ..    |
|                      | 2     | .. | .. | ..    | Nil   | ..    | N. T.  | ..     | ..    | ..    |
| 19- 2-36             | 1     | .. | .. | ..    | ..    | Nil   | Nil    | Nil    | ..    | ..    |
|                      | 2     | .. | .. | ..    | ..    | ..    | ..     | ..     | ..    | ..    |
| 4- 3-36              | 1     | .. | .. | ..    | ..    | ..    | ..     | ..     | Nil   | ..    |
|                      | 2     | .. | .. | ..    | ..    | ..    | ..     | ..     | ..    | ..    |
| 19- 3-36             | 1     | .. | .. | ..    | ..    | ..    | ..     | ..     | Nil   | ..    |
|                      | 2     | .. | .. | ..    | ..    | ..    | N. T.  | ..     | ..    | ..    |
| 11- 4-36             | 1     | .. | .. | N. T. | ..    | ..    | Nil    | N. T.  | N. T. | Nil   |
|                      | 2     | .. | .. | ..    | ..    | ..    | N. T.  | Nil    | Nil   | ..    |

\* N. T. = nil or traces.

of nicotine and the quantity of nicotine in the tobacco scion is very much reduced. The data are discussed briefly below.

*Tomato scions on tobacco stocks.*—On examining the results of periodical analyses in Table I it will be seen that in the tomato portion of the graft, there was no nicotine till 24-12-35, i.e., about 25 days after

making the graft, when it appeared in the young tomato leaves. In the older leaves it was first detected in the samples taken on 8-1-36.

In the old tomato stem periodical increases and decreases are to be noted indicating movement of nicotine from the tobacco portion below the graft and possibly transference to the other parts of the plant. This view gets support from the increase in the nicotine content of old leaves and by the appearance of nicotine in the young leaves of the scion (tomato) even as early as the last week of December.

Again, little or no nicotine was found in tomato fruits, but here it is not clear whether nicotine was used up in fruit development or spread over a larger tissue area. It is, however, certain that nicotine was not toxic to the tomato portion of the graft.

*Tobacco scions on tomato stocks.*—The results of analyses of these grafts detailed in Table II tell a different story. There was no downward movement from tobacco to tomato. Even in the tobacco scion the nicotine is too low to assume distribution over a larger area of the plant. It was probably used up in the elaboration and growth of the tobacco portion above the graft, in the flowering and fruiting processes. This is as would be expected, for as Theron and Cutler<sup>5</sup> observe, nicotine is a storage product which is drawn upon for elaborating material for growth or fruit formation or both. One of us has made similar observations in regard to the growth and development of the seed of *Cannabis indica*, where the oleo-resin is used up in the formation of seed.<sup>6</sup>

The results of the experiments conducted during the period 1934-36 were summarised in a paper read by us before the Indian Science Congress in January 1938.<sup>7</sup> It was then pointed out that "the tomato scions in grafts where tobacco was used as the stock distinctly showed the presence of nicotine suggesting upward translocation of the alkaloid manufactured in the tobacco stock, whereas in the reciprocal grafts the tomato stocks were devoid of nicotine and the quantity of the latter in the tobacco scions was very much reduced". It was also stated that nicotine content increased with maturity in the tobacco plants up to a stage after which there was a decline.

### 3. *Further Experiments with Grafts*

The experiments were interrupted in 1936 as a result of the transfer of the Imperial Agricultural Research Institute from Pusa to New Delhi. Work on this subject was resumed when in order to study further the translocation of nicotine, a subsidiary experiment was conducted during the winter of 1937-38. In this experiment plants of tobacco and tomato were

grafted together in pairs, the grafting being done laterally (*i.e.*, a longitudinal piece of the cortex was pared away from the stem of each plant for a distance of about three inches in the middle of the stem, and the two surfaces thus exposed were brought into close contact with each other and tied together in the same way as for other grafts) and both plants being allowed to remain *on their own roots*. When union was well established, after a period of about two months, the constituents of each graft were separated by gently tearing away the fused tissues at the line of union, and grown separately. Analyses for nicotine content were made on samples taken at the time of separation of the grafts and on leaf samples taken three weeks later, from two grafts. Leaves of non-grafted tobacco plants were also analysed.

The results are summarised below:—

| Tobacco leaves (non-grafted)       |    |    |    | Nicotine percentage       |                              |
|------------------------------------|----|----|----|---------------------------|------------------------------|
| 1st plant                          |    |    |    | 0.57                      |                              |
| 2nd plant                          |    |    |    | 0.63                      |                              |
| Tobacco leaves from grafted plants |    |    |    | At the time of separation | Three weeks after separation |
| (a) <i>Above graft</i> —           |    |    |    |                           |                              |
| 1st plant                          | .. | .. | .. | 0.63                      | 0.22                         |
| 2nd plant                          | .. | .. | .. | 1.04                      | 0.25                         |
| (b) <i>Below graft</i> —           |    |    |    |                           |                              |
| 1st plant                          | .. | .. | .. | 0.77                      | No leaves available          |
| 2nd plant                          | .. | .. | .. | 0.99                      | ..                           |
| Tomato leaves from grafted plants  |    |    |    | At the time of separation | Three weeks after separation |
| (a) <i>Above graft</i> —           |    |    |    |                           |                              |
| 1st plant                          | .. | .. | .. | Nil                       | 0.17                         |
| 2nd plant                          | .. | .. | .. | ..                        | Nil                          |
| (b) <i>Below graft</i> —           |    |    |    |                           |                              |
| 1st plant                          | .. | .. | .. | Nil                       | No leaves available          |
| 2nd plant                          | .. | .. | .. | 0.45                      | ..                           |

Although the number of samples is small, it is clear that nicotine or the substances necessary for its synthesis has moved laterally from the tobacco stem into the tomato stem and thence to the tomato leaves. In the case of the first plant nicotine could not be detected in tomato leaves at the time the grafts were separated, but it was present when the leaves were sampled three weeks later. The second plant did not show this, but as the quantities

present at this stage are small it is possible that traces were actually present but could not be detected. The results of analysis of the tomato leaves below the graft in the second graft are anomalous in that this is the only instance where nicotine appears to have travelled downward in the tomato plant.

The fact that in the first tomato plant nicotine could not be detected at the time of separation but was found three weeks later appears to indicate that nicotine is not necessarily translocated as such.

Our experiments are not yet completed and further work is contemplated. In view however of an interesting paper by Dawson<sup>8</sup> in which the results of similar experiments are presented and discussed it has been deemed desirable to publish the results obtained so far. A statement showing points of agreement and difference between our results and those of Dawson is given below:—

| Dawson's results   | Our results   |
|--|---|
| 1. When tobacco scions are grown upon tomato stocks no appreciable accumulation of nicotine in tobacco leaves or stems occurs.   | Do.   |
| 2. When tomato scions are grown upon tobacco stocks nicotine is found in small quantities in the tomato stems and fruits, and large quantities of the alkaloid accumulate in the leaves. | Do. as far as stems and leaves are concerned. It was doubtful whether or not fruits contained traces of nicotine.   |
| 3. Nicotine accumulates only in those grafts which possess a root system.  | Do. "Lateral grafts" (these were not attempted by Dawson) indicate that nicotine may appear sometime after the tomato portion has been separated.   |
| 4. In tobacco scions on tomato stocks only the basal portion of the scion contained the nicotine, the central and upper portions containing no traces.                                   | Young leaves (representing new growth) were analysed separately only once. On this occasion a value of 0.06 was obtained in one plant, the other showing no nicotine. A sample of older leaves analysed at the same time showed no nicotine at all. |
| 5. Lateral transport of materials across the graft union was found in "approach grafts".   | Do. in the case of our "lateral grafts".  |
| 6. A kind of leaf injury occurs when nicotine accumulates in tomato leaves.  | Not observed.   |
| 7. No evidence was found of downward movement of nicotine.   | Do., except in one case of a "lateral graft".   |
| 8. The author appears to think that nicotine is translocated as such and not as some precursor or intermediate.  | The development of nicotine in the tomato constituent of a "lateral graft" only some time after its separation does not support this assumption.  |

### *Discussion*

Carpenter,<sup>9</sup> Jurits<sup>10</sup> and Chamberlain and Clark<sup>11</sup> have shown that percentage nicotine in the tobacco leaf increases with maturity, while

Cutler *et al.*<sup>12</sup> found that in Turkestan tobacco (*N. rustica*) the nicotine content decreased if the plants were allowed to become over-mature. Earlier experiments by one of us (B. V. N.) have shown that tobacco seed and young seedlings are free from nicotine and that it is developed subsequent to the transplantation of the seedlings and increases until flowering time when it rapidly falls off in the period between flowering.

Statement showing nicotine content in different parts of the tobacco plant, at various stages of its growth:—

| Age of plant in days | Q nicotine in |        |      | Flower and fruit |
|----------------------|---------------|--------|------|------------------|
|                      | Leaf          | Stem   | Root |                  |
| 25                   |               | Traces |      | ..               |
| 45                   | 0.48          | ..     | 0.20 | ..               |
| 70                   | 1.50          | 0.22   | 0.35 | ..               |
| 92                   | 2.78          | 0.57   | 0.55 | ..               |
| 135                  | 4.50          | 0.51   | 0.50 | ..               |
| 170                  | 3.00          | 0.31   | 0.50 | ..               |
| 200                  | 1.69          | 0.20   | 0.30 | Traces           |

The material was grown in the Guntur area where sowing of seed is done at the end of August, and transplanting of seedlings in early October.

The increase of nicotine in the stem, root and leaves and its eventual decline suggest that it is a reserve product which is used up when the plant matures and begins to form seed. In the case of tomato scions grafted on tobacco stocks there appears to be similar rise and fall in the nicotine content although this is not so clear as in the case of the non-grafted tobacco plant. It would be interesting to follow what actually happens to the nicotine which is accumulated in the tomato scions; in the absence however of experimental data on this aspect of the problem, speculation would be idle and the drawing of conclusions must be deferred until more work on the subject is done.

#### Summary

1. The distribution of nicotine between stock and scion in reciprocal grafts between tobacco (*N. rustica*) and tomato (var. Pritchard) was studied. The tomato scions in grafts where tobacco was used as the stock distinctly showed the presence of nicotine in the stem and leaves. In the reciprocal grafts however the tomato stocks were devoid of nicotine and the quantity of the latter in the tobacco scions was very small.

2. Besides the cleft grafts referred to in 1 above, a number of lateral grafts were also made, the tomato and tobacco components being separated



FIG. 2. A graft with tobacco scion and tomato stock

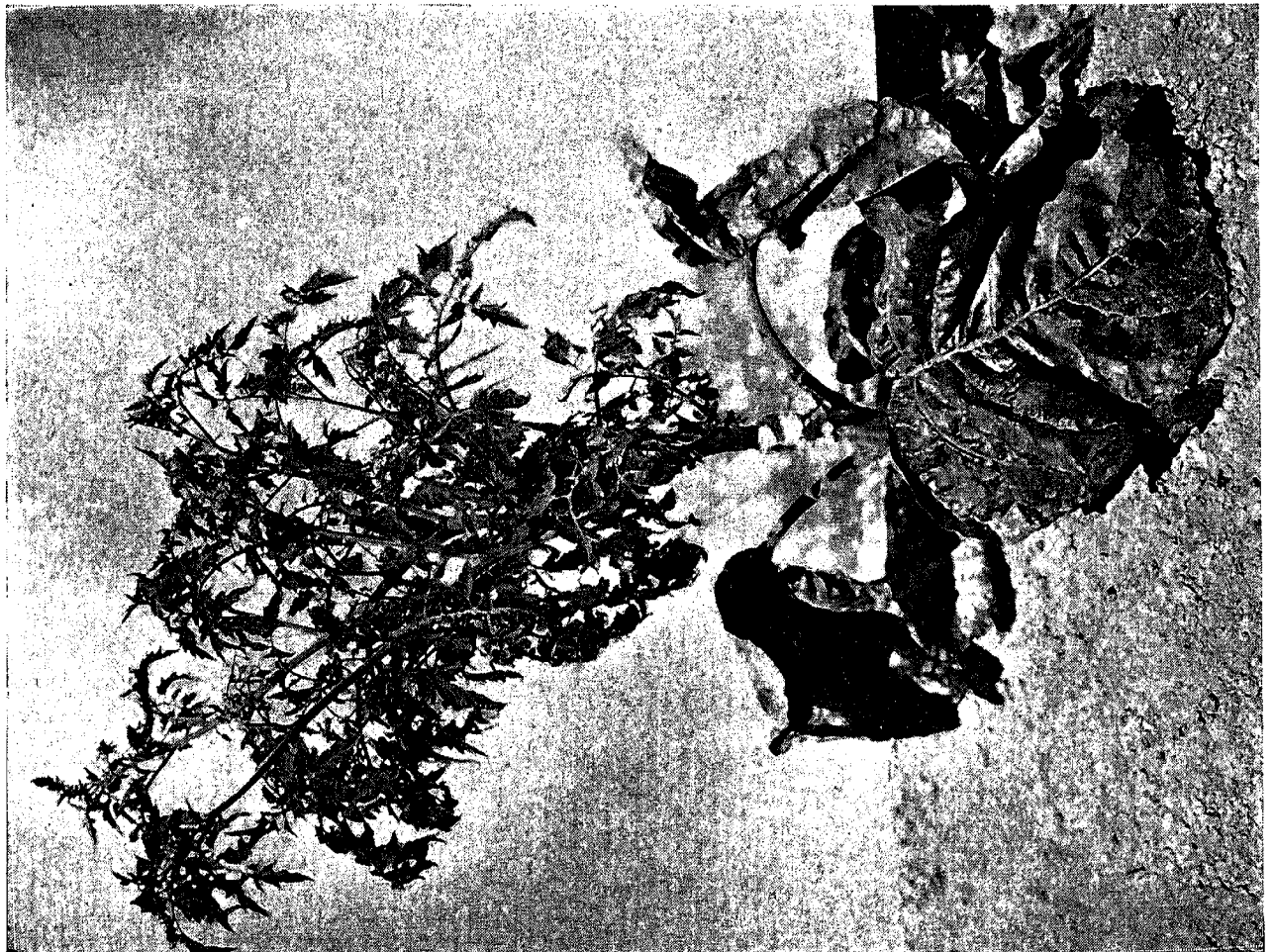


FIG. 1. A graft with tomato scion and tobacco stock

about two months after grafting and analysed at the time of separation and again three weeks later. In these grafts also there was evidence that nicotine had passed, in this case laterally, from the tobacco to the tomato.

3. Indication was found that nicotine content increases with maturity in tobacco plants up to a certain stage after which there is a decline.

4. The results obtained by Dawson in America are referred to and discussed in the light of the present data. The role of nicotine in the metabolism of the tobacco plant is also very briefly discussed.

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