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## TRILLIUM NIVALE.

## F. M. Andrews.

In the year 1898 the writer transplanted twenty specimens of Trillium nivale from the "North Pike" road, about one mile north of Bloomington, to his yard. The purpose of this was to be able to study more conveniently certain structural features and observe as closely as possible the period of anthesis over a series of years.

A study of the habitat was made and all of the observed conditions as to soil, shade and other factors were duplicated closely. It was necessary to protect the plants at first by a low box frame, but the sides of this box stood at some distance from the plants and afforded no more shade to the plants than the rocks which originally surrounded the place from which the plants were taken. This distance between the plants and the sides of the box is evident to some extent in the



Fig. 1. Photograph of part of bed of Trillium nivale.

accompanying photograph (Figure 1). This photograph shows only one corner of the box and was taken five years after the transplanting had been done.

The box was four feet square, made of one-inch poplar lumber, was twenty-five cm. deep and had no bottom. It was found necessary later on to protect the plants at times by galvanized iron wire netting having meshes about one-half cm. square.

The transplanting was done in early spring, care being taken to

remove with each plant or cluster of plants a large mass of the soil in which they were growing. The early blooming of Trillium nivale is well known. This often takes place before the snow has left the ground. The warmth generated by the plant at times melts the snow away in the form of a small well about the plant and entirely to the ground. The plants which were transplanted in the way indicated above finally grew very densely, as shown by Figure 1, and the earliest bloomers melted the snow in large areas. Being able to follow the course of development more closely and conveniently when planted in the box, it could easily be seen that sufficient warmth was generated by the young plants as soon as they had broken through the soil to melt the snow, which was often present to some extent, although this melting was not evident at all times on the surface. On removing the top layers of snow a dome-shaped space over and about the young plant of considerable extent was usually to be found.

In the part of the box shown in the protograph (Figure 1) between fifty and sixty plants in bloom may be seen by a careful count. This photograph represents about one-fourth of the whole area of the box. The blooming plants were fully as numerous all over the interior of the box as in the part shown in Figure 1. This would amount to something over two hundred blooms which had been produced by the original plants and by new plants which had grown since the first ones were planted.

Two of the plants were observed to have advanced the growing end of the rhizome considerably during the first ten years. This amounted to a movement of fifteen cm., or an average of 1.5 cm. per year. The rhizome, however, did not increase apparently in total length from the time of transplanting, since as it advanced at the growing end it died away at the other end and thus maintained about an even length. This agrees with the mode of life of many other similar subterranean plant structures.

These plants seem, although densely crowded, to have continually and rapidly increased for the first ten years. Since then and during the last five years the average increase in number has been small. The number of individuals, although still vigorous, seems to have become so great that all the available space for more individuals to grow in the space offered by the box has been reached and a balance in this respect established. The plants are all equally illuminated and all the other conditions equally favorable for all. It will be noticed in Figure 1 that the plants of equal age show differences as to size. Especially in some individuals the photograph shows a decided difference in the size of the flowers. This difference is, however, only slightly more pronounced than the writer has observed on the average on equal counts of individuals made in the field and under the original conditions.

The density as to numbers of individuals rarely if ever equals that shown by Figure 1 over the same area in the original habitat. A few individuals may, of course, come up close together, but this is only for a very few specimens, while an equal number as that shown by Figure 1 would be scattered over a much larger area, since much more space is available in all directions. This plant in its native habitat shows a

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preference not only as to soil and as to illumination, but also as to drainage. This, as indicated above, was taken into consideration in arranging the plants shown in Figure 1.

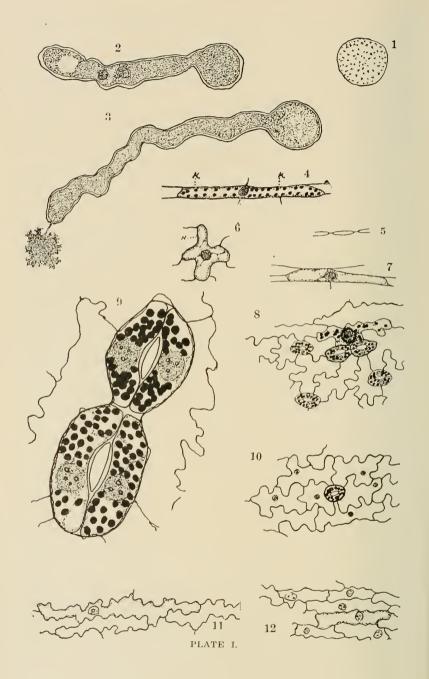
The blooming of Trillium nivale is not confined to the space of a few days, but in its native habitat, and as shown by the study of the plants partly illustrated by Figure 1, this period of blooming extended often several weeks, according, in part, to the condition of the weather, etc. The period of blooming was shortened by mild weather. The plants I transplanted showed a tendency to begin blooming somewhat earlier as a rule than those in the field, but this was slight.

Pollination was effected by bees which swarmed at times about the flowers, visiting first one and then another continuously. In this way an exceptional opportunity was afforded to watch in a small space and on numerous individuals the way the large amount of pollen was transferred from one flower to another. One insect in this way often visited a half score of flowers in a few minutes, so that the distribution of the pollen was thoroughly done. The pollen is produced in large amounts in each flower and the cells of the anther which open lengthwise down the margin allow the pollen to be puffed out somewhat and made easy of attachment to the visiting insect.

In only a few instances was there any tendency toward a monstrosity in Trillium nivale. This occurred in the transplanted specimens and was evidenced by a partial transformation of the petals in one of the flowers to leaves. This instance of phyllody in Trillium nivale, however, seems to be extremely rare and is all the more surprising when it is remembered that the genus Trillium is rather inclined to monstrosities in instances of phyllody in various of its species. The writer has called attention to some of these monstrosities in a former paper1 on three of the species of the genus Trillium, namely, Trillium sessile, Trillium recurvatum and Trillium erectum. These three species seem to be more susceptible to variation in this respect than is Trillium nivale. This one case of phyllody during the twentythree years these plants of Trillium nivale have been under direct observation and where from twenty-five flowers at first to about the two hundred flowers which were produced in 1921 shows that this tendency is rather unusual in this species, when successive yearly observations over a long period and finally in large numbers gave ample opportunity for its detection.

The pollen grains are nearly round and in surface view show short, blunt, numerous points about evenly distributed over the surface (Plate I, Figure 1). They germinate readily in solutions consisting of 3% cane sugar to which is added 1½% of gelatine. In such a mixture germination takes place to a considerable extent after two hours. This is shown in Plate I, Figure 2, where the pollen tube has attained a length of about three times the diameter of the spore. After three hours the pollen tube has grown to about four times or more the diameter of the spore on the average, and as a rule is considerably more irregular in outline, Plate I, Figure 3.

<sup>&</sup>lt;sup>1</sup>Andrews, F. M. Proceedings of the Indiana Academy of Science, 1905, pp. 187 and 188.



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At times when this length of pollen tube has been reached, it bursts, this always taking place at the end and often with considerable force so that the contents is generally forced out through a small pore and in a small stream (Plate I, Figure 3). The contents then collects as a ragged edged-mass at the end of the small stream of contents whose length is controlled by the force of expulsion, which is generally sufficient to force out the stream of contents to a length about equal to the diameter of the pollen tube (Plate I, Figure 3). When placed in distilled water the pollen grains of Trillium nivale frequently explode at once and apparently with considerable force, considering the size of the grain. This, of course, is due, as is known of some other kinds of pollen grains, to a rather sudden increase in the hydrostatic pressure of this cell. This, however, occurs in Trillium nivale as a rule in an unusually short period of time. Movement of the protoplasm of the pollen tube, as in Plate I, Figures 2 and 3, may often be seen.

The elongated epidermal cells of the stem are often filled with red colored sap and, partly due to rapid growth, have unusually thin outer walls for cells in such a position (Plate I, Figure 4). The nucleus is large in proportion to the size of the cell and several nucleoli are usually present. The red cell sap, when present, partly conceals the chlorophyll granules. The lateral walls of the epidermis even though very thin are provided with shallow pits (Plate I, Figure 4 K), which can just be observed when magnified 300 times. These pits, which are often seen in the walls of internal cells of many plants, are brought out with great definiteness when magnified 1,060 times. They are then observed to have rounded edges and are rather broad (Plate I, Figure 5 K).

The cells of the epidermis from the upper side of the leaf of Trillium nivale are wavy in outline and show no stomata. A slow movement of the protoplasm can generally be seen under favorable conditions in the various strands. The nucleus which is sometimes about central and sometimes parietal is rather large and shows in many cases several nucleoli (Plate I, Figure 6). Pits in the walls, as at H, Plate I, Figure 6, are barely discernible when magnified 200 times. The cells from the midrib on the upper side of the leaf of Trillium nivale show, as would be expected, a decided elongation and reduction in diameter. A large nucleus and rather actively moving protoplasm at times are generally much in evidence, the latter especially when magnified 450 times (Plate I, Figure 7).

The lower epidermis of the leaf of Trillium nivale shows, as usual, the presence of stomata. The chlorophyll granules are very few in number, but active movement of the protoplasm is often evident. The nuclei are rather large, often nearly equalling the diameter of some of the cells. The stomata, which are present on the lower surface of the leaves exclusively, are generally arranged so that one communicates with a respiratory cavity. Some departures from this arrangement are, however, present, as when two stomata are over one respiratory cavity as is shown in Plate I, Figure 8. The writer has shown this to be the case in a former paper. In the paper referred to, the history and

Andrews, F. M. Proceedings of the Indiana Academy of Science, 1914, pp. 209-211.

literature on this subject are given and the same peculiarity in some other plants is noted. As indicated in that paper, interesting questions concerning the location, development, and reactions of the supernumerary stomata await solution.

The outer epidermis of the sepal frequently shows twin stomata over one respiratory cavity<sup>2</sup> (Plate I, Figure 9). The strands of protoplasm often showed movement for 24 hours when magnified 450 times. The inner epidermis of the sepal, as the outer epidermis, has very wavy walls, but more pronounced than the outer epidermis in this respect (Plate I, Figure 10). Stomata are present, but few in number. In these cells the movement of the protoplasm often continued for as much as six hours during observation, as could be easily seen with moderate magnification.

The epidermis from the outside of the petal of Trillium nivale showed, as usual, much elongated and narrow cells and with very wavy thin walls (Plate I, Figure 11). The epidermis from the inner surface of the petal has smaller cells than those of the outer epidermis, its cell walls are much less wavy and more delicate (Plate I, Figure 12).

The above-mentioned facts coincide closely with similar observations made on specimens of Trillium nivale which were obtained from the native habitat. Certain other points, such as some of those above referred to, deserve further study, which can best be carried out under the conditions described in this paper.

## EXPLANATION OF PLATE I.

All of the figures of this plate are of Trillium nivale.

Fig. 1. Pollen grain, surface view x 300.

Fig. 2. Pollen grain after two hours in three per cent cane sugar plus one and one-half per cent gelatine x 300.

Fig. 3. Pollen grain after three hours in the solution used for the grain shown in Fig. 2 x 300.

Fig. 4. Epidermal cell from stem x 300.

Fig. 5. Piece of cell wall of cell illustrated in Fig. 4, showing pits at K enlarged x 1060.

Fig. 6. Cell from upper epidermis of leaf showing pits at H x 300.

Fig. 7. Cell from midrib of upper epidermis of leaf x 100.

Fig. 8. Few cells from lower epidermis of leaf x 100.

Fig. 9. Twin stomata from outside of sepal x 520.

Fig. 10. Few cells from inner epidermis of sepal x 100.

Fig. 11. Epidermal cells from outside of petal x 100.

Fig. 12. Epidermal cells from inside of petal x 100.

<sup>&</sup>lt;sup>2</sup> Andrews, F. M. Proceedings of the Indiana Academy of Science, 1914, pp. 209 and 210, Fig. 1.