

*Glomerata* in the Upper and Lower Austral.

*Umbellata* in the Tropical, Upper and Lower Austral.

*Obtusiflora* in the Tropical, Transition and Upper Austral.

*Salina* in the Transition and Boreal.

*Exaltata* is found only in the Lower Austral.

The above facts may be presented in tabular view as follows:

| Boreal.                | Transition.                                                                                                                           | Upper Austral.                                                                                                                                                                                 | Lower Austral.                                                                                                            | Tropical.                                                                                                                                                                                                                                  |
|------------------------|---------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Salina.<br>Tenuiflora. | Salina.<br>Tenuiflora.<br><br>Californica.<br>Subinclusa.<br><br>Obtusiflora.<br>Epithymum.<br>Epilinum.<br>Denticulata.<br>Rostrata. | Tenuiflora.<br>Glomerata.<br><br>Umbellata.<br>Obtusiflora.<br><br><br>Squamata.<br>Odontolepis.<br><br>Inflexa.<br>Applanata.<br>Cuspidata.<br>Compacta.<br>Decora.<br>Gronovii.<br>Arvensis. | Glomerata.<br><br>Umbellata.<br><br><br><br>Exaltata.<br><br>Cuspidata.<br>Compacta.<br>Decora.<br>Gronovii.<br>Arvensis. | Californica.<br>Subinclusa.<br>Umbellata.<br>Obtusiflora.<br><br><br>Squamata.<br>Odontolepis.<br><br><br><br>Potosina.<br>Palmeri.<br>Americana.<br>Corymbosa.<br>Tinctoria.<br>Jalapensis.<br>Mitreformis.<br>Floribunda.<br>Gracillima. |
|                        | Chlorocarpa.<br>Leptantha.                                                                                                            | Chlorocarpa.<br>Leptantha.                                                                                                                                                                     |                                                                                                                           |                                                                                                                                                                                                                                            |

#### NOTES ON THE GERMINATION AND SEEDLINGS OF CERTAIN NATIVE PLANTS.

BY STANLEY COULTER.

In the study of the phanerogamic flora of the State, some problems respecting the distribution or rather the non-distribution of certain species seemed to require for their solution somewhat extended germination experiments. These experiments have been in progress for three years, under conditions to be indicated later.

Incidentally the seedlings were carefully studied, more especially as to their resistance to temperature and moisture changes, in a less degree as to the form and arrangement of their earlier foliage leaves, since these, perhaps, in many cases may be regarded as representing inherited forms, while the later leaves stand for adaptive responses to light intensity and other ecologic factors. The results of the observations upon this point have not been sufficiently considered to warrant their presentation at this time, except in a few instances which indicate that many suggestions as to relationships would probably be one of the results of such a study.

It will be recalled, when we consider our native plants, that the increase in numbers and the consequent amount of territory occupied by any specific form bears no direct relation to the number of seeds it may produce. Indeed, the production by a given plant of a vast number of seeds, with adaptations for a wide dispersal, should, perhaps, be taken as an index of the intensity of its struggle for existence, and stand as a sure sign that, save through some change in ecological factors, the form will do little more than maintain itself in nature.

This view, which it will be remembered was advanced by Weismann, is being confirmed by observations upon plants. The setting of a large number of seeds stands not as the sign of a rapid increase in numbers of the form, but rather the reverse. An example or two may emphasize the statement.

The common nightshade (*Solanum nigrum*) is a plant with which all are familiar. Where it obtains a foothold it usually holds its own, but rarely becomes dominant or so increases in number from year to year as to attract attention. From one of these plants which bore forty-three berries and was still flowering, I took three berries and planted them after having broken the outer walls. *One hundred forty-two* seedlings appeared. Surprised at the result I planted three other seedlings similarly treated, being especially careful to eliminate error. In this case *one hundred eighty-seven* seedlings appeared. This would indicate that each berry contained on an average at least fifty viable seeds, and as there were over forty berries, the potential product from that single plant was over two thousand plants.

From the ordinary *Scrophularia*, germination percentages ran from fifty-six to seventy in the favorable conditions of the laboratory, indicating an almost incredible possible increase from a single plant. Yet every

botanist knows that this plant makes no visible increase in numbers from year to year. The great number of capsules filled with seeds is but the sign of its intense struggle.

In many cases the causes which hold in check the undue increase of a specific form are evident. The law requiring that stock should be kept within bounds, modified in very marked degree the flora of the State, and in certain regions has served to cover stripped hills with a new timber growth. But there are cases in which the number of seeds produced is so enormous, the means of dispersal so various and ingenious, and the dispersal itself so sure, that we wonder that the increase is no more rapid. In this category we find notably the composites. Considering their myriads of seeds and their perfect means of seed dispersal, their increase in numbers is insignificant. Indeed, in many cases special protective devices have been developed in order that they may maintain their place in nature.

It is evident that factors other than those ordinarily limiting plant distribution are operative in limiting the distribution of the composites, or that the composites are peculiarly sensitive to conditions which do not materially affect the majority of plants. The theory of special limiting factors seemed scarcely worthy of consideration. It was therefore presumed that some, at least, of the ordinary factors were more effective than in other cases, and that in all probability there was a peculiar sensitiveness to these changes at some particular stage of development. The experiments were undertaken with the hope of throwing some light upon these points. The conditions of the experiments have been varied from time to time as experience suggested, and to such an extent as to preclude tabulation in the limits of this paper. Conditions may be summarized as follows: Two kinds of soil were used—a loam mixed with sand and a leaf-mold. In both cases the soil was carefully sifted and packed in the pots in order to prevent subsequent settling. As regards moisture, three conditions were used—saturated, moderately moist and extremely dry. The percentage of water in the soil was not carefully worked out, but was roughly estimated to range from 25 per cent. in one extreme to between 80 and 90 per cent. in the other. Planting was either surface or at about the depth of the seed. The average temperature in the first series of experiments was 26.5° C., with extremes of 20° C. and 31° C. In the second and third series the average temperature was 24° C. As far as could be determined by inspection only perfect seeds were used. As a matter of course, control experiments served as checks upon results.

From the results of the experiments the following conclusions seem deducible.

FIRST.—*The germination percentage in the composite is low as compared with that of the other families examined.*

This statement has its apparent exceptions in *Arctium Lappa* and *Cnicus lanceolatus*, which show high percentages. That these are exceptional, however, will be shown later. From the experimental cards the following table is drawn:<sup>1</sup>

|                                             | <i>Per cent.</i> |
|---------------------------------------------|------------------|
| <i>Arctium Lappa</i> .....                  | 93.3             |
| <i>Cnicus lanceolatus</i> .....             | 89.3             |
| <i>Bidens bipinnata</i> .....               | 37.5             |
| <i>Bidens frondosa</i> .....                | 30.0             |
| <i>Lactuca Canadensis</i> .....             | 37.5             |
| <i>Lactuca Scariola</i> .....               | 25.0             |
| <i>Solidago Canadensis</i> .....            | 12.5             |
| <i>Anthemis cotula</i> .....                | 12.5             |
| <i>Cnicus muticus</i> .....                 | 10.0             |
| <i>Aster Shortii</i> (one year).....        |                  |
| <i>Ambrosia</i> (one year).....             |                  |
| <i>Vernonia fasciculata</i> (one year)..... |                  |

Germination percentages shown by forms in other families are as follows:

*Solanum nigrum*, 187 seedlings from three berries.

|                                      | <i>Per cent.</i> |
|--------------------------------------|------------------|
| <i>Datura Tatula</i> .....           | 100.0            |
| <i>Abutilon Avicennae</i> .....      | 89.0             |
| <i>Scrophularia nodosa</i> .....     | 70.0             |
| <i>Plantago Rugellii</i> .....       | 62.5             |
| <i>Rumex Acetosella</i> .....        | 56.7             |
| <i>Malva rotundifolia</i> .....      | 45.0             |
| <i>Capsella Bursa-pastoris</i> ..... | 45.0             |
| <i>Nepeta Cataria</i> .....          |                  |
| <i>Chenopodium album</i> .....       |                  |

<sup>1</sup> These are taken from over 200 experimental cards and are fairly typical of the series. Experiments with ordinary germinating apparatus confirmed these results.

These percentages are not exceptional, having been repeatedly obtained. I have not been able to secure germination in *Chenopodium*, a fact possibly due to the late collection of the material.

SECOND.—*In most cases the achenes show the highest germinating percentage if collected at about the middle of the flowering season.*

If, for example, the flowering period is from July to October, achenes collected in the latter part of August or first of September will give a higher germinating per cent. than those collected at any other season. This is true in all forms studied, with the exception of *Arctium*. The non-viability of the later achenes may be explained as due to the action of frost. That of the earlier achenes is not so readily explained. In *Arctium* the achenes show a ready viability at all seasons. In the case of *Bidens* it is possible that the later achenes might show a fair percentage of viability, as no late collections of this genus were made. The difference in viability at different floral periods does not seem to exist in the other families studied. On the contrary, in the case of *Abutilon* and *Solanum*, the highest percentages were obtained from the seeds collected very early in the season.

THIRD.—*For the most part the central achenes of the head are not viable, and the same condition is frequently found in the outer rows.*

In all cases the largest germination percentage was obtained from achenes taken about midway between the center and periphery of the head. An exception to this statement is perhaps found in *Helianthus*, in which, in a single experiment, the central achenes were found to show a high germination percentage. A consideration of the order of maturing of the flowers in the composite head taken in connection with the mode of pollination furnishes the probable explanation of this fact.

FOURTH.—*The seedlings of all composite forms studied, were found to be particularly sensitive to both temperature and moisture changes.*

Very slight changes in either of these conditions, especially if sudden, proved fatal almost without exception. An increase in temperature of 5° C., brought about in thirty minutes and continued for three hours, killed all the seedlings of *Bidens*, *Cnicus*, *Lactuca*, *Solidago* and *Anthemis*, the only composite seedlings escaping being those of *Arctium*. In the case of the other forms, *Scrophularia* alone succumbed, *Abutilon*, *Malva*, *Solanum*, *Datura* and *Capsella* not being visibly affected nor apparently retarded in their growth.

A similar temperature change, brought on gradually (three hours) and continued for three hours, only produced about a 40 per cent. fatality among the composites.

Changes in moisture, either in the soil or in the air, found a ready response in the behavior of the composite seedlings. A diminution of moisture, which would not even produce wilting in the seedlings of other families, would not infrequently prove fatal to those of the composites. The effect of moisture increase was not so readily seen, although there exists under such conditions a tendency on the part of the stem of the seedling to rot near the ground, a tendency apparently shared by *Abutilon*, and which seems about the only check put upon the increase of this latter form.

As might be expected *direct sunlight* works against the seedlings of composites, as, indeed, against all others, but with a peculiarly fatal effect in these sensitive forms. It has been found necessary in all cases to protect them from the direct sunlight for several days, usually until after the development of the first foliage leaves. Such extreme sensitiveness was rare, if at all present in the other families studied.

FIFTH.—*Cotyledon leaves of nearly related forms closely resemble each other, a resemblance often carried on in the earlier true leaves.*

The cotyledon leaves of *Arctium*, *Cnicus lanceolatus* and *Cnicus muticus* are almost exactly alike. The only dissimilarity observable being that in *Arctium* the green is a trifle darker than in *Cnicus*. The resemblance is so exact that I discarded the first germination experiments with *Cnicus*, supposing that through inadvertence *Arctium* achenes had been planted. With the appearance of the first true leaves *Arctium* is plainly marked off from *Cnicus*, but the two species of *Cnicus* are not to be separated until the appearance of at least the third foliage leaf. By this statement I mean that I think that at this point I can detect the beginnings of the leaf characters of the forms. The same conditions are found in the *Lactucas*. *Scariola* cannot be separated from *Canadensis* in any of the seedling stages so far as my observations go, indeed, though I carried the seedlings through the development of the seventh foliage leaf I found no marked indication of specific foliar differences. I think, without multiplying instances, that in very many cases supposed relationships between the species of a large genus, and certainly between many genera, might find corroborative evidence in a study of the early foliage of the seedlings.



SIXTH.—*In most cases the highest germination percentages in composites are obtained from surface planting in leaf mold.*

Arctium is the exception to this statement also. By surface sowing upon leaf mold, under the three conditions of moisture, Arctium gave an average germination percentage of 22.2 per cent. In sandy loam, where the achenes were covered by sifting earth over them, the percentage rose to 93.3 per cent. In all other cases, however, the leaf mold gave much the larger per cent. This was especially marked in the case of *Cnicus lanceolatus*, in which in sandy loam the percentage was 12.5, while in leaf mold the average percentage was 89, rising in one case to 100 per cent. The experiments indicate strongly that successful germination in composites is more closely dependent upon the character of the soil than is the case in the majority of our native plants. In other words, their soil range is so narrow that a slight modification in its character may practically prevent germination of the achenes and thus very effectually limit distribution.

SEVENTH.—*The water content of the soil does not (so far as is indicated by these experiments), affect the germination percentage to the degree that might have been expected.*

While differences in the water content of the soil affects the seedlings in a marked way, germination is unaffected under a wide range in water-content of the soil. In extreme cases, some forms show marked variations. Arctium, for example, which gives a germination percentage of 93.3 under ordinary soil moisture conditions, falls to 3.3 per cent. in the case of extreme dryness. *Cnicus lanceolatus*, on the contrary, rises to 100 per cent. in extremely dry soil. In the forms studied the highest percentage of germination is found under moderately moist conditions, and the lowest under extremely dry conditions, save in the case of *Cnicus lanceolatus*, where the reverse seems to be true. Extreme soil moisture in many cases leads to the moulding of the achenes.

A careful dissection of material used in these experiments showed that there was nothing in the character of the achenes themselves to which this low germination percentage could be attributed. The material was collected with care expressly for these experiments, and dissection demonstrated well formed embryos in more than 80 per cent. of the achenes examined.<sup>2</sup> I am, therefore, led to believe the low germination

<sup>2</sup> The achenes thus examined were taken from the portion of the head indicated in conclusion three, *supra*.

percentages to be due to changes in external conditions, to which, perhaps, the forms are peculiarly responsive. The ease and certainty with which high germination percentages were secured in other families certainly lends support to the view.

The experiments are still in progress, as there are still many points to be worked out in detail. Among them are the effects of varying soil temperatures, of a wider range of soils, of progressive experiments for the determination of resting periods in the various forms, of duration of viability, of the effect of freezing, and others self-suggestive to the experimentalist. Until these are worked out in detail the question as to the causes of the relatively small distribution of any given composite form must remain open. So far as the experiments go they point to this limitation being due in a very large degree:

1. To a low germination percentage, largely due to an extreme sensitiveness on the part of the embryo to external conditions, to which should perhaps be added imperfect pollination, due to causes already given.

2. To an extreme sensitiveness of the seedlings to temperature and moisture changes, either in soil or atmosphere. This necessarily brings about a peculiar sensitiveness to direct sunlight.

When the habits of most of our native composites are considered it will be seen that this extreme sensitiveness in both achene and seedling proves an effectual limitation to their distribution. Other factors than these here emphasized enter, but none are of such general application.

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#### FORMALIN AS A REAGENT IN BLOOD STUDIES. BY ERNEST I. KIZER.

Among the most common reagents used in the demonstration of blood corpuscle structure, are found osmic acid, salt solutions, picric acid and acetic acid. But all of these cause distortions of the corpuscles, so they are imperfect fixing agents and preservatives. The method of drying blood on the coverslips is seldom successful in the hands of beginners.

Formalin has been found very useful in this connection, both as a fixing agent and as a preservative, because it produces no appreciable dis-