

INFLUENCE OF EXTERNAL FACTORS ON THE ALKALOID CONTENT IN SOME MEDICINAL PLANTS

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The form in which a plant appears at a given moment depends not only on its genetic constitution (14, 33, 38, 42) and its stage of development (12, 18, 30, 32), but also on the external conditions under which it has grown. It is known that the external conditions can have a modifying effect on the external and internal characters of a plant. The external conditions are provided by climate and soil, which both consist of a complex of separate factors each of which, theoretically, may be considered capable of affecting growth to a greater or lesser extent.

On the basis of these experiences and expectations one might want to create for a certain crop such external conditions as are the most favourable for growth and the production of active principles. Before doing so, one had to find out experimentally which of the climate and/or soil factors have the greatest effect on growth and the production of active principles.

Climate and soil, however, are magnitudes of which most factors are not readily modifyable and controllable. The climatic factors can only be controlled in air-conditioned glasshouses, whereas the influence of the soil is best studied by means of pot trials.

1. Soil factors.

A factor that can fairly easily be modified is the richness of the soil in mineral nutrients. Much was expected from the influence of this external factor. Hence research on modification has largely consisted of manurial trials (2, 4, 5, 7, 10, 13, 16, 17, 19, 20, 25, 27, 29, 36). Mainly the influence was studied of the elements nitrogen, phosphorus, potassium and calcium on growth and alkaloid content of various alkaloid containing crops. These elements were administered in different quantities and compounds.

The results from these experiments, which were usually carried out in the open, are both with us and with other research workers rather disappointing and confusing and often diametrically opposed. Some investigators obtained a favourable effect from a certain fertilizer in a certain alkaloid containing crop. Others, however, obtained a negative effect. Moreover it was found that various alkaloid containing crops respond differently to the same fertilizer. This makes the situation still more confused.

If, nevertheless, one wants to draw conclusions from the experiments made, it may be said that a dressing of nitrogen generally

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stimulates growth. It makes a difference, however, in what form this dressing is given. Manuring with the elements phosphorus, potassium and calcium often has a much less marked effect on growth, probably due to the soils not being equally rich in these elements and to differences in the capacity of the plants to take up these elements.

From the trials it was shown that manuring with these elements often had a favourable effect on growth. In many cases, however, no effect could be observed and in some cases, mainly when the applications of fertilizer were too heavy, or in unfavourable compounds the effect was even unfavourable.

The effect of all these elements on the alkaloid content is still less pronounced and it is altogether impossible to arrive at a uniform conclusion.

In my opinion it is of the utmost importance that the soil on which the trial is to be carried out should be analyzed first. Manuring can then be done in accordance with this analytical report. It would be better still to make pot trials in an air-conditioned glasshouse using soils of which the composition is known.

In addition to manuring with inorganic fertilizers attempts have been made to influence the production of alkaloids by manuring with organic compounds, viz. the so-called precursors, hormones and antibiotics. The precursors can be considered as building bricks for the alkaloid synthesis. Manuring was done via the leaf and/or the root. Dressings via the leaf seem to have had no effect, whereas manuring via the root with the amino acids arginine, ornithine and putrescine showed a favourable effect in *Atropa* and *Datura* (6, 28, 34). Other amino acids, however, had no effect. Certain hormones and antibiotics (1, 43) seem to influence both the alkaloid content and yield. This research is only in its initial stage.

The influence of other soil factors were hardly investigated.

2. Climatic factors.

The influence of some climatic factors was investigated but hardly ever as an isolated factor. One had to content oneself with outdoor trials, taking by-effects of other factors into the bargain (24).

The factor light has in some cases a favourable effect on growth and the alkaloid content, in other cases this factor acts as a check on both. (9, 15, 41). This probably depends on the fact whether the crop under investigation was a "light-loving" or a "shade-loving" one.

The temperature probably shows an optimum both for growth and the production of alkaloids, again dependent on the crop (11).

Precipitation generally decreases the alkaloid content owing to the alkaloids being washed out of the plant (39, 40).

In the course of years various authors have published reviews of this subject, to which they mostly added a conclusion. Some of these conclusions follow hereafter.

K r e y e r (31) is of opinion that irrespective of the nature of the environmental conditions under which the alkaloid containing plants

have grown, they always cause an increase in alkaloid content as soon as they act as a check on plant growth, and that they cause a decrease in this content as soon as they stimulate growth.

R o m e i k e (35) and H e c h t (21) believe that growth and alkaloid content are less influenced by environment than by genetic factors and the development stage. J a i m e N o s t i N a v a (26), after having made extensive manurial trials with plants containing active principles concludes: "Fertilizers have no reliable effect on the active principles of these crops. Selection would be a better means of increasing the alkaloid content". D a f e r t and H i m m e l - b a u e r (8), who for 25 consecutive years made manurial trials with various crops, obtained no marked effect on growth and alkaloid content. B o s h a r t (3) determined that differences in alkaloid content caused by fertilizers fluctuate between limits that are, pharmacologically, hardly important. R o w s o n (37) on the other hand says that a good combination of fertilizers stimulates growth and that mainly nitrogen in the form of ammonium sulphate, combined with phosphatic fertilizers, increases the alkaloid content.

Finally I will cite H e g n a u e r (22, 23) who says "Variation in experimental material, ontogenetic differences and the absence of an absolutely reliable basis for calculating the analytical results are factors which make a correct interpretation of the modification experiments very difficult. Variation in material can be eliminated by using chemical strains or clones. Ontogenetic differences must be eliminated by harvesting the crops in the same stage of development. A better basis for calculation than the dry matter would be e.g. the crude fibre-content or the leaf-surface".

It is also my opinion that, if one should stick to this, a great improvement of investigational practices would be achieved.

In addition it might be useful to devise a certain scheme for the investigations of fluctuation. Wider international collaboration in the form of simultaneous research in certain field is also advisable.

LITERATURE REVIEW

1. B e a l, J. L. et al., *J. Am. pharm. Assoc.* 43, 282 (1954).
The effect of selected chemicals on the alkaloidal yield of *Datura tatula* L. In some cases they obtained an alkaloid-increasing effect in *Dat. tatula* from hormone sprays on the leaf as well as on the soil.
2. B o s h a r t, K., *Heil- und Gewürzpflanzen* 2, 76 (1918—19).
Der Anbau einiger medizinisch wichtiger Solanaceen.
At the time he cited from literature contradictory manuring effects in *Atropa belladonna*.
3. B o s h a r t, K., *Heil- und Gewürzpflanzen* 18, 79 (1938—39).
Neue deutsche Arbeiten über Düngung im Arzneipflanzenanbau.
In the course of one day the alkaloid content may fluctuate considerably. Therefore harvesting must always be done at the same time of the day. The weather, too, can have a prolonged effect on metabolism in the *Atropa* plant.
It was determined that manuring has only a slight effect on the alkaloid content. The differences in content fluctuate between limits that are hardly important from a pharmacological view point. Growth, however, is affected by manuring.

4. Brandt, Ch., *Ber. schweiz. bot. Ges.* 61, 67 (1951).
Beitrag zur Kenntnis der chemischen und morphologischen Eigenschaften einiger Lobelia-Arten im Zusammenhang mit Anbau- und Selektionierungsversuchen (Diss.)
Nitrogen is said to cause an increase in yield but a decrease in the alkaloid content. Potassium did not affect yield but had a favourable effect on the alkaloid content.
5. Brewer, W. R. et al., *J. Am. pharm. Assoc.* 39, 586 (1950).
The effect of nutritional and soilreaction fertilizers on the production yield and total alkaloidal content of *Atropa belladonna* and *Hyoscyamus niger*.
According to these authors the elements nitrogen and phosphorus and a high pH have a favourable effect on weight, while the elements nitrogen and potassium cause an increase in alkaloid content. These results were only obtained in *Atropa belladonna* and not in *Hyoscyamus niger*.
6. Cromwell, B. T., *Biochem. J.* 37, 717 (1943).
Studies on the synthesis of hyoscyamine in *Atropa belladonna* L. and *Datura stramonium* L.
Arginine, hexamine, putrescine and formamol, either with or without glucose in the feeding solution, had a favourable effect on the alkaloid content.
7. Dafert, O. et al., *Heil- und Gewürzpflanzen* 14, 98 (1931-32).
Düngungsversuche mit *Datura stramonium* L. und *Hyoscyamus niger* L.
They made manurial trials with the elements N, P and K. Manuring increased weight but did not affect the alkaloid content. Studying the influence of the elements separately did not lead to uniform conclusions. In order to determine the influence of manuring, one would have to eliminate the effect of all other factors, mainly the climatic factors.
8. Dafert, O. et al., *Die Landeskultur* 3, 147, 163 (1936).
These investigators made manurial trials for 25 years and came to the conclusion that nitrogen increases weight in *Datura stramonium* but does not affect the alkaloid content. Only very heavy N-dressing may also cause an increase in content. K, P and Mn seem to have an adverse effect on the alkaloid content. *Atropa belladonna* and *Hyoscyamus niger* react to manuring in the same way as *Datura*.
9. Deluard, H., *Bull. Sci. Pharm.* 30, 11 (1923).
De l'influence des radiations solaires sur le développement de la belladonna et sur sa teneur en alcaloïdes.
Plants grown in full sunshine had about three times as much foliage and about twice as much alkaloids as plants grown in a shaded place.
Consequently light had a clearly favourable effect.
10. Dijkstra, S. P. De invloed van Kalkammonsalpeter op de bladopbrengst en het alkaloidengehalte van *Datura stramonium* var. *inermis*. Diss. Groningen 1950.
The fresh yield increased, while the alkaloid content in the fresh material remained the same. The dry matter percentage decreased, causing the alkaloid content calculated on dry matter to increase.
11. Elzenga, G. et al., *Euphytica* 5, 276 (1956).
Influence of the temperature on growth and alkaloid content of first-year *Atropa belladonna* L.
The temperature had little or no effect on plant height and weight of dry matter in corresponding developmental stages. Both growth and alkaloid content were highest at 23° C, then at 26° C and finally at 20° C. The differences in alkaloid content were slight but mathematically reliable.
12. Elzenga, G. et al., *Euphytica* 5, 259 (1956).
Interrelation of alkaloid content and stage of development of 1- and 2-year old *Atropa belladonna* L.
When judging the plants for alkaloid content it is important to harvest all plants in the same stage of development, since this content is not steady during growth.
13. Elzenga, G. et al. Bemestingsproeven met *Atropa belladonna* (not published).

N-dressings increase weight and tend to decrease the alkaloid content. This effect, however, is not mathematically reliable. K-dressings had a varying effect both on growth and alkaloid content. P-dressings also produced varying effects on yield and alkaloid content. The pH did not affect yield, while a low pH had a favourable effect on alkaloid content. This effect was not always reliable.

14. Elzenga, G. et al. De alkaloidevorming van *Atropa belladonna* (not published).
100 young *Atropa* plants were cut into halves. These halves, called A and B components, were placed in pots separately. The soil mixtures in the pots were identical, and the plants were grown in an air-conditioned glasshouse. At the harvest there were still great differences in alkaloid content between the A and B components, although the differences between genetically identical plants were smaller than the differences in alkaloid content between plants that were genetically different. A reliable positive correlation exists between the ratio root weight shoot weight and the alkaloid content in the shoot.
15. Elzenga, G. et al. Invloed van het licht en de temperatuur op het alkaloidgehalte en de opbrengst van *Atropa belladonna* (not published).
High temperatures and additional illumination give high yielding plants with a low alkaloid content. The alkaloid content is a little higher if growth is checked somewhat by a lower temperature. It is highest at a high temperature in the dark. In that case the shoot hardly grows whereas the root makes normal growth, causing an accumulation of the alkaloids in the shoot. At a low temperature in the dark the alkaloid content is a little lower, probably due to the life-process being retarded at low temperatures in the root as well.
16. Elzenga, G. et al. Invloed van de hoeveelheid en de aard van de stikstofbemesting op de opbrengst en het alkaloidgehalte van *Lobelia inflata* (not published).
Neither the amount nor the nature of nitrogen dressings had any effect on yield or alkaloid content of *Lobelia inflata*.
17. Esdorn, I. *Heil- und Gewürzpflanzen* 19, 1 (1940).
Untersuchungen über den Alkaloidgehalt von *Lobelia inflata* L. in Abhängigkeit von äusseren und inneren Faktoren.
In general plants on plots manured with N, P, K and Ca grew better than those on untreated soil, but their alkaloid content was lower.
18. Evans, W. C. et al. *J. Pharm. and Pharmacol.* 5, 772 (1953).
Changes in the ontogenetic production of alkaloids in *Atropa* and *Datura*.
The alkaloid content depends on the stage of development of the plant. In 1-year-old plants the alkaloid content is at first low; it is highest when the plants are in flower, and decreases again after flowering. In 2-year plants the alkaloids content is at first high, decreases until flowering, remains practically steady during flowering and decreases afterwards.
19. Flück, H., *J. Pharm. and Pharmacol.* 6, 153 (1954).
The influence of the soil on the content of active principles in medicinal plants.
According to this author N-dressings increase the alkaloid content of *Atropa belladonna*, *Hyoscyamus niger*, *Datura stramonium* and *Datura innoxia*. N in the form of NO_3 is said to decrease the alkaloids in *Lobelia inflata*. P-dressings increase the alkaloid content in *Datura* and *Chelidonium*, but had no effect on *Atropa* and *Lobelia* and an adverse effect on *Hyoscyamus*. K-dressings increased the alkaloid content in *Lobelia* and *Hyoscyamus* and had an adverse effect on *Atropa belladonna*, *Datura stramonium* and *Datura innoxia*.
20. Gstirner, F., *Pharmazie* 5, 498 (1950).
Düngungsversuche mit *Atropa belladonna*.
The elements nitrogen, phosphorus and potassium had a clearly favourable effect on weight; nitrogen and potassium also had a strong effect on the alkaloid content.

21. Hecht, W., *Heil- und Gewürzpflanzen* 14, 15 (1931—32).
Bioklimatische Versuche zur Erforschung der Ursachen der Gehaltsschwankungen bei Arzneipflanzen.
The alkaloid content is influenced by the following factors, in order of importance: 1. the species, 2. the stage of development, 3. the climate, 4. the soil.
22. Hegnauer, R., *Pharm. Weekblad* 88, 1, 37, 106 (1953).
Over de invloed van externe factoren op het gehalte aan werkzame bestanddelen van geneeskrachtige planten.
There is no general agreement on the influence of external factors on the production of active principles. At the same time there are diverse opinions as to the importance of each separate factor for the quality of the medicinal plant when harvested.
This is often due to a misinterpretation of the trial results. When studying the influence of external factors it is, of course, of the utmost importance to eliminate the much stronger effects of the internal factors by using varieties or clones. Unfortunately, these are not always available. Plants grown under different conditions often occur at the same moment in a different physiological stage. For a good comparison it is therefore necessary to harvest the plants in the same physiological stage.
Calculating the alkaloid content on the dry matter is only of relative value, as the percentage dry matter can show fluctuations of 20% within 24 hours.
23. Hegnauer, R., *Pharm. Weekblad* 89, 505 (1954).
Over de invloed van de groeiplaats op de eigenschappen van geneeskrachtige planten.
It is highly essential that in modification experiments chemical strains should be used as starting-material. In order to be able to interpret correctly the environmental effects on the synthesis of secondary materials, it is necessary to harvest the plants at the same developmental stage. Variation in plant material, ontogenetic differences and the absence of an absolutely reliable basis for calculation of the analytical results are factors which make a correct interpretation of modification experiments very difficult.
Variation in material can be eliminated by using chemical strains or clones. Ontogenetic difference can be prevented by harvesting the plants at the same stage of development. A better basis for calculation than the dry matter e.g. is the crude fibre-content or the leaf-surface.
24. Hemberg, T. et al., *Pharm. Acta Helv.* 28, 74 (1953).
Die Tagesperiodizität des Alkaloidgehaltes und des Gehaltes an übrigen stickstoffhaltigen Stoffen bei *Datura stramonium* L.
The alkaloid content varies in 24 hours. It decreases from 7 to 23 hours and increases again from 23 to 7 hours. The difference in alkaloid content may amount to 22% if calculated on dry crude fibre weight.
25. Hoffmann, F. H., *Ber. Schweiz. bot. Ges.* 59, 285 (1949).
Über den Einfluss einiger Bodenarten auf Wachstum und Gehalt von Arzneipflanzen.
Nitrogen and phosphorus increase the alkaloid content in *Atropa belladonna* and *Lobelia inflata*, whereas K causes a decrease in alkaloids. The pH had no effect, while a high percentage of sesquioxides in the soil had an adverse effect on alkaloid content.
26. Jaime Noste Nava, D. et al.
Trabajo equipo presentado por la seccion de farmagoergasia del Instituto del C.S. de I.C. Estudios Arcera de la limitada influencia de los abonados, sobre la produccion de principios activos por las plantas medicinales.
The authors made extensive manurial trials with *Atropa belladonna*, *Datura stramonium*, *Lobelia inflata* and many other medicinal plants. They arrived at the conclusion that fertilizers had no economic effect on the content of active principles in the plants investigated. Selection is said to offer better possibilities of increasing the alkaloid content.
27. James, G. M., *Econ. botany* 1, 230 (1947).
Effects of manuring on growth and alkaloid content of medicinal plants.
Nitrogen had a very favourable effect on growth and alkaloid content of

- Atropa belladonna*, but the form in which nitrogen was applied played an important part.
P, K, Ca and Mg had no effect, while P and K in too high concentrations tended to produce an adverse effect.
28. James, W. O., *New Phytol.* 48, 172 (1949).
The amino-acid precursors of the Belladonna alkaloids.
The application of precursors such as the amino-acids l-arginine and l-ornithine had a slight, but mathematically reliable, alkaloid-increasing effect. Other amino-acids had no effect.
 29. Manske, R. H. F. and H. L. Holmes. *The Alkaloids* Vol. 1 New York, Academic Press 1950, 525 pp. Chapter II. W. O. James. Alkaloids in the plant 15-90.
According to this author fertilizers have an effect both on growth and alkaloid content.
N in the form of NO₃ promotes growth but does not effect the alkaloid content. N in the form of NH₄ arrests growth, causing the alkaloid content to increase. Ca, P and high pH have a favourable effect on the alkaloids. Increasing light and darkness cause an increase in alkaloids. The later effect is probably not real l-arginine and other amino-acids increase the alkaloid content.
 30. Jentzsch, K., *Sci. pharm.* 21, 285 (1953).
Beitrag zur Kenntnis der Alkaloidbildung in Solanaceen.
Datura stramonium var. *inermis* and *Datura tatula* mainly have scopolamine in their juvenile stage and hyoscyamine when they are older. In various alkaloid containing plants not only the total content but also its composition is variable during plant growth.
 31. Kreyer, G. K., *Heil- und Gewürzpflanzen* 13, 1 (1930-31).
Ertragshöhe und Drogenqualität von Arzneipflanzenanbau.
In the case of *Datura*, manuring promotes growth but decreases the alkaloid content. *Atropa* is a shade-loving plant and consequently makes worse growth and has the highest alkaloid content when grown in the light. *Datura* is a light-loving plant and consequently makes worst growth and has the highest content when grown in the shadow. In view of this the author comes to the following general conclusion:
„Irrespective of the nature of the environmental conditions surrounding an alkaloid containing plant, these conditions always cause an increase in alkaloids as soon as they arrest growth, while they cause a decrease in alkaloids when they have a growth-promoting effect.“
 32. Lie m, S. G., et. al, *Pharm. Weekblad* 90, 513 (1955).
Pharmacognostische onderzoeken met zeven vormen van *Datura stramonium*.
Apparently small differences in developmental stage may result in a highly variable scopolamine content.
 33. Führer des Max-Planck Institutes für Züchtungsforschung, 33, (1953).
Datura tatula — polyploidie.
The tetraploid plants had about three times as much alkaloids as the diploid ones.
 34. Reinouts van Haga, P.
Bijdrage tot de kennis der biosynthese van de alkaloiden bij *Atropa belladonna* L. Diss. Delft (1956).
The amino acids arginine, ornithine and putrescine increase the rate of growth and the alkaloid content, which effect is still further increased by adding 2-4% saccharose to the feeding solution.
 35. Romeike, A., *Pharmazie* 8, 668, 729 (1953).
Beiträge zur chemischen Physiologie der mydriatisch wirkenden Solanaceen-Alkaloide.
Atropa belladonna contains more scopolamine in its juvenile stage than later. With increasing age *Hyoscyamus niger* and *Datura stramonium* continue to contain more scopolamine than *Atropa belladonna*. The genetic factors have a greater influence on the alkaloid content than the environment.

36. Rowson, J. M., *J. Pharm. and Pharmacol.* 2, 201 (1950).
 „The pharmacognosy of *Atropa belladonna* Linn.”
 Ca, K and P have little effect on yield and alkaloid content as compared with the marked effect of nitrogen.
 To obtain a maximum alkaloid content it is necessary to harvest parts of plants that are making active growth.
37. Rowson, J. M., *Pharm. Tijdschr. België* 31, 97 (1954).
 Alkaloidal biogenesis in plants with special reference to *Datura* and allied genera in the Solanaceae.
 A good combination of fertilizers increases the yield by weight. Nitrogen, especially in the form of ammonium sulphate combined with phosphatic fertilizers, increases the alkaloid content.
 Tetraploid plants of *Atropa belladonna*, *Datura stramonium* and *Hyoscyamus niger* are higher in alkaloids than the diploids.
38. Rudolf, W. et al., *Planta* 39, 36 (1951).
 Polyploidie-effekte bei *Datura tatula*.
 The alkaloid content was markedly higher in all parts of the tetraploid plant than in the diploid one. It was notably in the leaves that the difference became apparent.
39. Sandfort, E., *Angew. Bot.* 22, 1 (1940).
 Über die Ursachen der Schwankungen im Alkaloidgehalt bei *Datura stramonium*.
 Yield and alkaloid content were highest on soils rich in nutrients. The chemical constitution of the soil is more important than its structure and texture. Temperature and rain are the most important climatic factors.
 The alkaloid content increases with an increase in temperature, whereas the alkaloids are washed out by rain.
40. Schratz, E., *Die Deutsche Heilpflanze*, 8, 69 (1942).
 Über den Einfluss des Regens auf den Alkaloidgehalt des Stechapfels, *Datura stramonium*.
 Rain of a certain duration and violence can have a strong effect on the alkaloid content as the alkaloids, in particular those in the leaf, are washed out. A positive correlation exists between the amount of rainfall and loss of alkaloids.
41. Stuljnikoff, M., *Heil- und Gewürzpflanzen*, 12, 23 (1929-30).
 Neue Russische Arbeiten über Arzneikultur.
 Leaves of plants that had grown in broad daylight contained twice as much atropine as those of plants that had grown in the shadow.
42. Steinegger, E., *Pharm. Act. Helv.* 27, 311 (1952).
 Untersuchungen über die Vererbung des Alkaloidgehaltes bei *Datura*.
 The alkaloid content of *Datura* is genetically determined. By crossing tetraploid plants that are high in alkaloids and subsequent selection it must be possible to obtain high-grade selections.
43. Steinegger, E., *Pharm. Act. Helv.* 29, 256 (1954).
 Der Einfluss von Antibiotica auf Wachstum und Wirkstoffgehalt von *Datura*.
 Penicillin had a very favourable effect on growth and yield, but an adverse effect on the percentage of alkaloids in the fresh product.