

INTERRELATION OF ALKALOID CONTENT AND STAGE OF DEVELOPMENT OF 1- AND 2-YEAR-OLD *ATROPA BELLADONNA* L.

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INTRODUCTION

Successful selection is primarily dependent on a thorough knowledge of the characters of the crop under selection. When selecting *A. belladonna* for high alkaloid content we established that in one and the same plant this content was far from constant during the course of growth. Hence the question arose in which stage the plants contain the highest alkaloid content.

BREWER and HINER (1) found the highest content in *A. belladonna* during flowering. The British Pharmacopoeia (3) probably agrees with this, since it requires that the plant shall be harvested during flowering. On the other hand CARR (2) did not find any significant differences in the alkaloid content at the different stages of development, except that the concentration decreases rapidly when the leaves are dying. EVANS and PARTRIDGE (6) encountered the highest concentration in 1-year-old *A. belladonna* during flowering. The concentration falls markedly during fruit setting. At first the alkaloid content of 2-year *A. belladonna* is high, but later it decreases rapidly until flowering begins, remains steady during flowering and after that decreases further.

ROWSON (8), in his paper on research on *A. belladonna*, emphasizes the importance of collecting actively growing aerial parts to obtain a maximum content of 1-hyoscyamine.

METHOD OF INVESTIGATION

In order to reduce any possible influence of soil differences on the alkaloid content, each trial was duplicated. Each block consisted of 25 rows of 10 plants each. The plant material used had not been selected. The trials were laid out in a sandy soil, which was yearly manured with 41.5 kg. N, 43.7 kg. P_2O_5 and 84.2 kg. K_2O per acre, and received in addition 24.3 ton stable manure every other year.

The leaves, stems or herb (the whole aerial part of the plant) were harvested twice a week, always at about 10 a.m. Each time 1 row of each block was harvested, dividing the row into 3 groups of plants (2 groups of 3 and 1 group of 4 plants). These groups were harvested separately. After the material had been weighed, cut up fine and mixed, a sample was taken and dried at 55-60°C. Then the total alkaloid content in the air-dry material was determined by the method of DIJKSTRA (4). The means of the 6 analyses per harvesting date were plotted in graphs. We shall discuss our findings from these graphs.

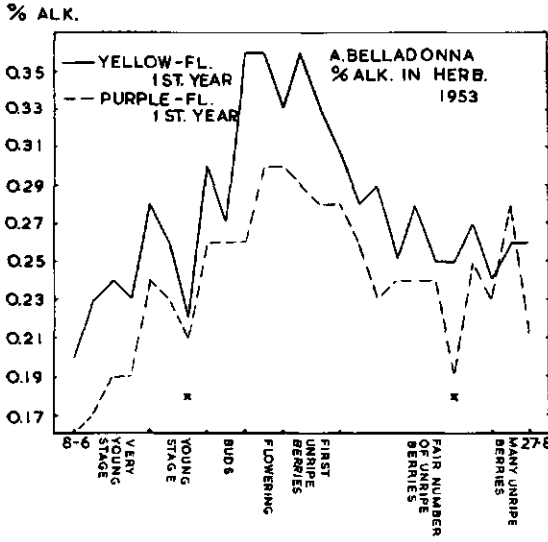


FIG. 1.
COURSE OF THE ALKALOID CONTENT OF
1-YEAR-OLD BELLADONNA HERB (1953)

EXPERIMENTAL RESULTS

The course of the alkaloid content in 1-year-old A. belladonna during growth

On May 22, 1953, two fields were planted with 1-year-old yellow-flowered and 1-year-old purple-flowered plants of *A. belladonna*, the two kinds well apart from each other. Harvesting was started in a very young stage on June 8. The last harvest was carried out on August 27 when the plants already bore many berries.

Figure 1 clearly shows that the total alkaloid content increases during growth until flowering begins and remains steady until the first berries appear. After that it drops

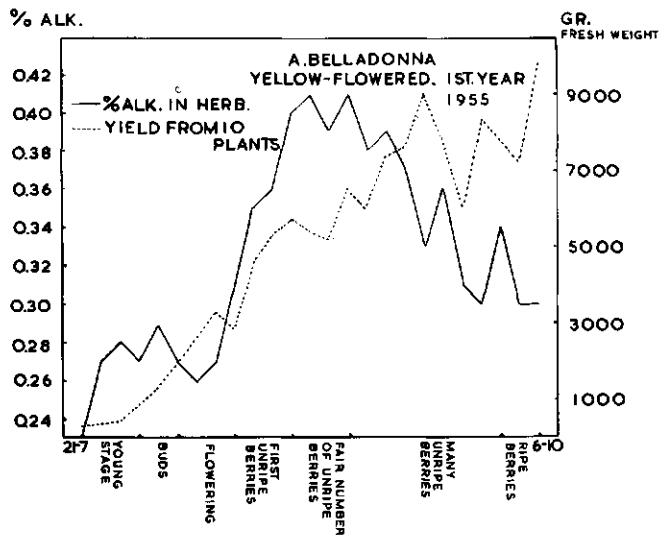


FIG. 2.
COURSE OF THE ALKALOID
CONTENT AND YIELD OF 1-
YEAR-OLD BELLADONNA HERB
(1955)

ALKALOID CONTENT AND DEVELOPMENT OF ATROPA BELLADONNA L.

again. Although the overall level of alkaloid is lower in purple-flowered plants, both varieties show parallel changes in alkaloid content during growth. The marked decreases (x) in alkaloid content which occasionally occur cannot be explained from the weather conditions (temperature, cloudiness, precipitation), which were equable during those periods.

The course of the alkaloid content in 1-year-old yellow-flowered *A. belladonna* was again determined in 1955. Owing to the unfavourable spring weather harvesting could not be started until July 21, i.e. 6 weeks later than in 1953. As is shown in Fig. 2, the changes in 1955 are very much the same as in 1953; only the maximum is a little higher and is reached somewhat later, when the plant has already produced several unripe berries.

The weight of the aerial parts increases regularly, even when the plants bear ripe berries. Too small a number of replicates may account for the irregular course of the weight curve.

Figs. 1 and 2 demonstrate that the maximum alkaloid content can be shifted to some extent.

From the experiences of EVANS and PARTRIDGE, BREWER and HINER, and from data which we collected in 1953, we may conclude, however, that the maximum alkaloid content in 1-year-old *A. belladonna* is generally reached during flowering and remains steady during the first fruit setting. But afterwards there is a decrease.

The course of the alkaloid content in the leaf is entirely different from that in the stem (Fig. 3). The alkaloid content of the stem, as compared with that of the leaf, is very high in the first growing stages. As in the leaf it reaches a maximum during flowering, but as the plant grows older it falls to the level of the alkaloid content in the leaf. Also the content in the stem of the purple-flowered variety is at first higher than that of the leaf. The level of the concentration in the stem is appreciably lower than that of the yellow-flowered variety.

As the weight ratio leaf/stem in young *A. belladonna* is approximately 3:1, the

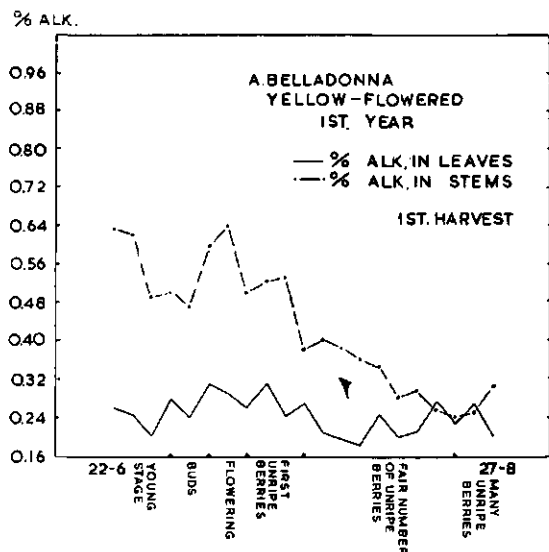


FIG. 3. COURSE OF THE ALKALOID CONTENT OF LEAVES AND STEMS OF 1-YEAR-OLD ATROPA BELLADONNA

alkaloid of the young herb is mainly determined by the leaf. As the plant grows older this ratio more and more approaches the value 1:1.

The course of the alkaloid content in 2-year-old A. belladonna during growth

Since *A. belladonna* is a perennial it can be harvested for a number of consecutive years. Now it might be asked whether the course of the alkaloid content in the 2-year-old plants runs parallel to that in the 1-year plants, and whether roughly the same overall level is reached.

The course of the alkaloid content in 2-year old yellow-flowered *A. belladonna* was studied in 1953 and in 1955. As stated above, the same procedure was followed as for 1-year-old *A. belladonna*. Contrary to observations by CARR we found that the alkaloid content in the leaf clearly shows a minimum when the plant begins to flower, and a maximum when the plant bears a number of green berries (Fig. 4). The level of the alkaloid content in the leaf of 2-year-old plants is higher than in 1-year plants. The same is true of the level in the stem. One-year plants were high in alkaloids in the young stage of the shoots, but this was even more markedly the case with 2-year plants. Also with 2-year plants the content in the stem falls with the age of the shoots to the level of the concentration in the leaf. This level is reached when the plants bear ripe berries.

If leaf and stem are not harvested separately the course of the alkaloid content is as shown in Fig. 5. The course of the content of the herb in the bud stage is largely determined by the quantity in the stem at that stage (Fig. 4). A low level is reached when the plant begins to flower, after which the content increases again to a maximum which is reached when the plant bears a good many green berries. At that stage the content of the leaf and that of the stem are about equally high. After that the content drops steadily until the berries ripen off.

Fig. 5 confirms the findings of EVANS and PARTRIDGE that the content in the aerial part of 2-year-old plants is at first very high. Later, however, the content takes

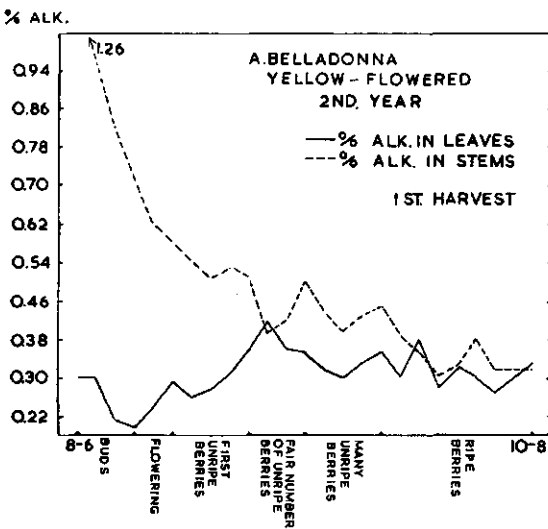


FIG. 4. COURSE OF ALKALOID CONTENT OF LEAVES AND STEMS OF 2-YEAR-OLD ATROPA BELLADONNA

ALKALOID CONTENT AND DEVELOPMENT OF ATROPA BELLADONNA L.

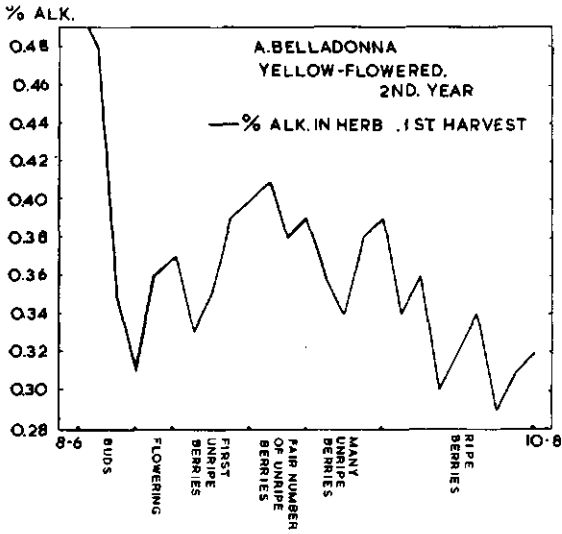


FIG. 5. COURSE OF THE ALKALOID CONTENT OF 2-YEAR-OLD BELLADONNA HERB (1ST HARVEST, 1953)

a different course from that described by the above-mentioned workers (see Introduction).

The second harvest

Two-year-old *A. belladonna* can be harvested twice in one season if the first harvest is carried out in time, i.e. shortly after flowering.

Let us now consider the course of the alkaloid content of the plants that have resumed growth. The conditions of light and temperature are now different from those prevailing at the first growth.

The first series of harvests was carried out from the end of May to early in August,

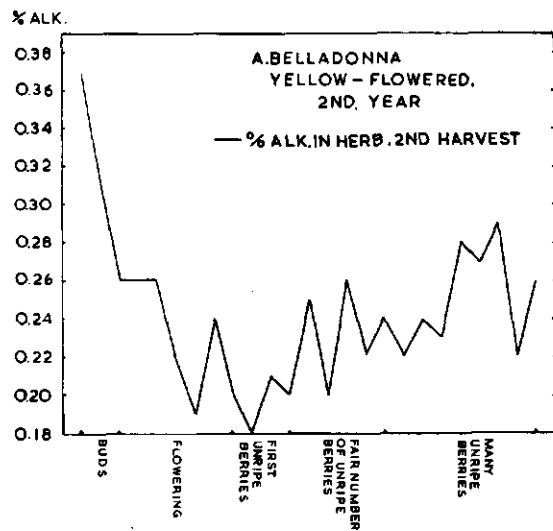


FIG. 6. COURSE OF THE ALKALOID CONTENT OF 2-YEAR-OLD BELLADONNA HERB (2ND HARVEST, 1953)

while the whole field was harvested again on September 8, but now at one time. Due to the difference in time required by the plants to resume growth between the first and the second harvest, all developmental stages were present on September 8, except the stage in which the plant already bears ripe berries. So analyses for alkaloids in plants in that stage could not be carried out. The rows that were harvested first already bore many unripe berries, while those that were harvested last had only just come into bud.

Again we found a relatively high alkaloid content in the young stage, and this was again mainly due to the stem (Fig. 6). The subsequent changes in alkaloid content during the resumed growth are practically the same as those in shoots of the first harvest, except that the maximum is reached at a later stage, namely when the plants already bear many berries. This is mainly due to the leaves, as the content in the leaves also increases steadily until that stage is reached. As the plants grow older the content in the stem steadily decreases to the level in the leaves. Plants from the second harvest were lower in alkaloids than those from the first harvest.

DISCUSSION

If the extent to which a certain character of a plant manifests itself at a given moment depends not only on genetical factors but also on the environment and the stage of development reached by the plant at that moment, selection for the character in question becomes extremely difficult. This is often the case with plants, e.g. herbs, that are important for their content of some valuable substance.

For a number of years we have been selecting *A. belladonna* for high total alkaloids in the aerial parts. SIEVERS (9) is of the opinion that the alkaloid content of *A. belladonna* is determined genetically, and STEINEGGER (10) produced evidence that this is correct for *Datura stramonium*, a plant that contains the same alkaloids as *A. belladonna*. ROMEIKE (7) claims that the alkaloid content is primarily determined by genetical factors and that the environment has only a slight effect.

An alkaloid content of 0.3 % in the dry matter of the herb of *A. belladonna* is normal, while a content of 0.6 % is high. The relative difference is 100 %, whereas the absolute difference is 0.3 %. As the selector is only concerned with such small absolute differences, it is of the utmost importance to him to know the extent to which the content, at a given moment, is determined by the genotype, the environment and the stage of development of the plant.

Like ROMEIKE we are of opinion that the environmental factors have only a slight effect, provided that there are no extreme differences and perhaps with the exception of trace elements. We will come back to this in a following publication dealing with the influence of temperature on growth and alkaloid content of *A. belladonna* (5).

We found that the interrelation of stage of development and alkaloid content is more important than the influence of the environment. It appeared that the relative differences in the alkaloid content of a plant from the same sowing could amount to over 100 %, while in the stem differences up to about 250 % may occur.

When the plants are harvested at frequent intervals it becomes apparent that the course of the content is more or less systematic. It is characterised by abrupt decreases and increases, which cannot be explained from the state of the weather. They may be related to the growth rhythm of the root and the aerial part, but this would have to

ALKALOID CONTENT AND DEVELOPMENT OF ATROPA BELLADONNA L.

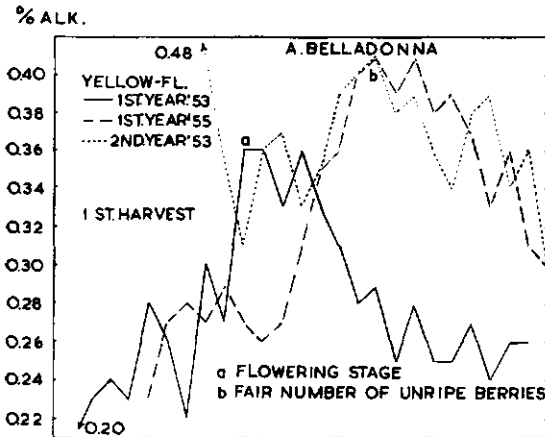


FIG. 7.
COURSE OF THE ALKALOID CONTENT OF
1- AND 2-YEAR-OLD BELLADONNA HERB
(1953 AND 1955)

be investigated further. These abrupt decreases and increases do not occur in the experiments carried out by EVANS and PARTRIDGE, probably because their harvest intervals were still too great. The selector can take advantage of this systematic course of the alkaloid content by carrying out all analyses at the same stage of development.

The stage at which the maximum occurs may shift to a slight extent (see Fig. 7, which is a combination of Figs. 1, 2 and 5). This shift might be due to the cold spring of 1955 when growth was much slower than in 1953.

The question put in the introduction might be answered by saying that for purposes of selection or when studying the influence of environmental factors 1-year-old *A. belladonna* can be best judged when the plant flowers and/or bears the first unripe berries, whereas 2-year-old *A. belladonna* should be judged when the plant already bears a fair number of berries. At this time it is possible to obtain information about the maximum alkaloid content.

SUMMARY

A study was made of the course of the alkaloid content in the aerial parts of both 1- and 2-year-old *A. belladonna* during growth. This content is not steady. One-year plants reach the maximum content during or just after flowering, when the plant bears a few green berries, while 2-year plants reach the maximum content in a very young stage. During flowering, however, the content drops to a low level to reach a peak again when the plant bears a good many green berries.

When judging the plants for alkaloid content it is important to harvest all plants in the same stage of development. If they are harvested at one time it would be certain that all plants would have been influenced by the same environment for the same length of time. However, a critical comparison of the potentialities of the different plants would not have been achieved.

SAMENVATTING

Het verband tussen het alkaloiidegehalte en het ontwikkelingsstadium van 1- en 2-jarige Atropa belladonna L.

Voor de veredeling van *A. belladonna* op alkaloiidegehalte is het belangrijk, dat alle planten in hetzelfde ontwikkelingsstadium geogst worden. Door de planten gelijktijdig te oogsten zou men wel de zekerheid hebben dat ze gedurende eenzelfde tijd door hetzelfde milieu beïnvloed zijn, maar dit zou een kritische vergelijking van de verschillende planten ten opzichte van het gehalte dat ze kunnen bereiken onmogelijk maken.

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¹⁾ Zolang de voorraad strekt kunnen deze publikaties franco worden toegezonden, na ontvangst van het vermelde bedrag op giro no. 425340 van het Instituut voor de Veredeling van Tuinbouwgewassen, S. L. Mansholtlaan 15 te Wageningen onder vermelding van wat verlangd wordt; ook bestaat de mogelijkheid deze publikaties uit de bibliotheek van het I.V.T. te lenen.

²⁾ Eerder verschenen publikaties zijn vermeld achterin in de Mededelingen nos 1 t/m 89.