

Research history

All over the world cocklebur species cause severe problems mainly in row crops, but their damage can be considerable on pasturelands and as invasive species in natural communities as well. Therefore, several authors study the biology, competitiveness and the regulation potentials of the *Xanthium* genus.

The competitiveness of cocklebur species has mainly been studied in maize and soybean, as these are the two largest cultures endangered by cocklebur; however, there is little information on its competition with other cultivated plants.

As cocklebur spreads in Hungary, such an investigation on other cultures (e.g. sugar beet, sunflower) would be useful.

Besides the widespread examination of competition ability, a relatively less intensively studied area is the allelopathy of cocklebur, although its existence has been identified for a long time. Therefore, the role of allelopathy in the competitiveness of the species has not yet been clarified. The actual role of allelopathy is controversial not only in cockleburs, but in other allelopathic weed species as well. The reason for this is the fact that allelopathy is influenced by several environmental factors and their changes affect research findings as well.

In order to make the research findings on allelopathy comparable and usable, the circumstances of experiments and their description should be focused on more factors than earlier and actually effective factors should also be selected out of them.

Plant protection against cocklebur species started very early with their natural enemies, but it has not yielded complete success yet. Out of organisms likely to be suitable for regulation, *Puccinia xanthii* (Schwein.) is one of the most intensively investigated species. Following its appearance in Hungary, its pathogenic capacity for cocklebur populations and its use against common ragweed has become reasonable. Moreover, we need to clarify if it can damage sunflower hybrids which are cultivated in Hungary.

Research goals

The topic of the paper is the competitiveness of Italian cocklebur, including one of its factors, allelopathy; the potential role of *Puccinia xanthii* Schwein. species, which is one of the potential organisms suitable for biological defence, in suppressing cocklebur species.

Our research goals were the following:

- Besides early emerging cocklebur, to examine the survival, the production capacities and the competitiveness of later emerging Italian cocklebur in sugar beet and maize cultures, so that we can get information from what stage of development the given cultures can prevent the seed formation in long-emerging cockleburs and cope with their yield reducing effects.
- To investigate allelopathy, one of the most difficultly conceivable factor in the competition between cultivated plants and weeds, against Italian cocklebur. In our investigation we studied several factors which influence allelopathy. The identification of their effects plays a significant role in allelopathy studies, in the comparison of the research findings and in the use of these findings.
- To survey the rate of infection by *Puccinia xanthii* in Hajdú-Bihar county, to investigate the rate of infection on cocklebur and to identify the occurrence and pathogenic capacity of the rust species for ragweed (*Ambrosia artemisiifolia* L.) and for cultivated sunflower hybrids (*Helianthus annuus* L.).

Methods of research

Investigation on the competition ability of Italian cocklebur

Competition examinations were performed in small-plot experiments (10 m²), with four replications, where we studied the competition between cocklebur and sugar beet, cocklebur and maize in 2002-2004.

We examined the survival and competition abilities of early and later emerging cockleburs as well. Studies on sugar beet showed that out of later emerging cockleburs, the first one emerged at the 5-10 leaf stage of sugar beet (its emergence took place 35-40 days following that of sugar beet), the second one in another 2-3 weeks and the third one in further 2 weeks, in a density of 3.3 pieces/m². In maize, out of later emerging cockleburs, the first one emerged 20 days following the emergence of maize (at the 6-7 leaf stage of the crop), the second one emerged in another 2-3 weeks and the third one in further 2 weeks, in a density of 3.3 pieces /m² and 15 pieces/m², respectively. The plot was free of other weeds, the cover values of the weed and the crop, in maize fields the height of the crop as well, were measured three times and we performed yield measurement at the end of the vegetation period.

Study on allelopathy in biotests

The allelopathy of Italian cocklebur was studied in biotests, for which we used fresh and dried (at 60 °C for 48 hours) weed sprouts and roots separately. We used the remnants of sprouts and roots separately and in a mixture as well. During the preparation of extracts, for 100 ml of solvent

we used fresh sprout or root of 4, 8, 16, 20g, or the respective amount of dried parts.

The applied solvent was a mixture of tap water (pH 7.1) and ethanol (96%). Extraction took place at room temperature for 24 hours in darkness. The test plants used in biotests were garden cress (*Lepidium sativum* L.), sugar beet (*Beta vulgaris* L.), spring barley (*Hordeum distichon* L.) and maize (*Zea mays* L.), which were germinated in Petri-dishes (diameter: 11cm). We evaluated the germination, root and sprout growth of sugar beet, as well as the root and sprout growth of the other three test crops.

Research focused on the production of cockleburs in different circumstances, which are used for the preparation of extracts, on the various dates of sample taking and on the effects of several factors from different biotest circumstances on allelopathy.

We examined the following influences of factors affecting allelopathy: the means of lighting during donor plant production, the development stage of donor plants, the density and water supply of their population, the effect of precipitation, the preparation of samples for extraction (using fresh, dried or ground plants) and the type of the extracting agent.

The quantitative measurement of allelochemicals

Besides germination trials, we followed-up the quantitative changes in cinnamic acid derivatives, which are known as allelochemicals detected earlier from cockleburs, i.e. chlorogenic acid, coumarin, p-coumaric acid and trans-cinnamic acid.

For the quantitative determination of the 4 compounds we prepared an extract from dried crop samples with distilled water, using a sample of 4 g

and distilled water of 100 cm³, which was shaken for 2 hours. After filtering, the agents were identified with Merck-Hitachi HPLC equipment.

The circumstances of separation were the following:

column: Lichrospher 100RP-18, 125x4mm;

12:15:1 mixture of eluent: water: methanol: acetic acid;

flow: 1ml/minute.

Detecting was performed with a L-4500 Diode Array Detector at a wave length of 275 nm. For quality identification we used a comparative solvent liquid containing chlorogenic acid (SIGMA), p-coumaric acid (SIGMA), coumarin (SIGMA) and trans-cinnamic acid (ALDRICH).

The quantitative identification of allelochemicals took place in the Regional Agro-Instrument Centre, Centre of Agricultural Sciences, University of Debrecen.

The gained values were compared with the findings of biotests.

Study on *Puccinia xanthii*

We studied the occurrence of *Puccinia xanthii* on cockleburs, sunflower and ragweed in 2002-2004. We identified the rust species on the morphology of pustules observed with unaided eye and that of the teliospores observed by light microscope.

In the identification of the rate of infection we examined 100 randomly selected crops, to find out if at least one pustule could be found on them; following this we counted leaves which contained and which did not contain telio-pustules. We counted the quantity of pustules per leaf in the case of 200 leaves, and then we measured the surface area of the leaves.

For the identification of the average pustule size, we measured each pustule on 30 leaves (with mm precision).

We monitored the potential infection of ambrosia in the mixed population of cocklebur and ambrosia. The populations were examined every week from the middle of July.

The occurrence of the rust was examined on 15 hybrids in a greenhouse and on fields. The 15 hybrids were the following: Zoltán, Hysun-321 PR, Alexandra PR, Rigasol PR, PR 63 A 90, NS-H-909, NS-H-930, NS-H-919, NS-H-703, NS-H-474, NS-H-901, NS-H-906, NS-H-927, NS-H-928, NS-H-923. We observed the development of infection on the fields and in the greenhouse as well. On the fields, sunflower hybrids were sown in small plots (10 m²) next to the population of cockleburs on 28 April, and also into flower pots at the same time and on 13 June, which we put directly next to the cocklebur plots. From the middle of July we monitored the emergence of pustules on cockleburs and on sunflowers once a week. We monitored the susceptibility of hybrids at 24 °C, at a relative humidity of 95-100% on 4-6 leaf sunflower entities, by “leaf disc method” in the greenhouse from the end of July (when pustules appeared on cocklebur leaves). We changed the leaf discs on a weekly basis.

In the evaluation of the findings we used the program package of SPSS 9.0 for basic statistics, for a single-variant variance analysis and for the examination of correlation and regression. In the single-variant analysis of variance we compared samples at the 5% significance level, identified correlations in our correlation examination at the 1% and 5% significance levels.

Main research findings

The competitiveness of Italian cocklebur

We examined the competition of cockleburs with cultivated crops in sugar beet and maize in the different dates of cocklebur emergence. In the case of sugar beet, the later emerging cocklebur (3.3 pieces/ m²), which appeared after the first wave of emergence, following the cultivated crop by 30-40 days, was competitiveness: although their initial development was rather slow, they overtook the sugar beet by August, reducing its yield and sugar content.

However, cockleburs emerging in another two weeks died at a young age; therefore, as opposed to earlier emerged weeds, they could not enhance either their seed supply in the soil, nor could they affect sugar beet yields negatively.

In maize, the first emergence of the weed, with the growth of its density (5-60 pieces/m²) reduced the yield proportionately, but even the least weed density resulted in a considerable loss of crop yield. Out of later emerging cockleburs, only those ones could significantly reduce maize yields, which emerged within 4 weeks following the cultivated crop; however, cockleburs were able to produce seeds even if they emerged 5-6 weeks following the emergence of maize. The competitiveness of early and later emerging cockleburs, thus enhancing the findings by other authors in maize and soy, was greater in years with considerable precipitation than in drier years. This also manifested in the height and seed production of the weed at the end of the vegetation period.

The allelopathy of Italian cocklebur

We tested the allelopathy of Italian cockleburs grown in fields and in greenhouses on cress, maize and spring barley.

Extracts prepared from the fresh and dry parts, the root and sprout remnants of Italian cocklebur affected the test plants and their vital processes (germination, growth) in a different way; however, all test crops verified the allelopathic effect in the performed biotests. Treatments affected the germination and growth of sugar beet, whereas they influenced only the growth of the other three test crops.

However, the rate and means of allelopathy depended not only on the species of the test crop, but on other factors as well, which supposedly influence the production, secretion and solubility of allelochemicals.

- The way and rate of the effect changed depending on the used plant pieces.
- Biotest results were determined by the fact, whether extracts were prepared from live plants or from plant remnants.
- The effect was influenced by the solvent (water, ethanol) on certain test crops.
- Extracts prepared from the same parts of plants grown in fields and in greenhouses resulted in different effects.
- The inhibitory effects of extracts prepared from cockleburs grown in greenhouses also depended on the fact, whether the weeds were produced in short or long day circumstances.
- The effects of cocklebur grown in fields were affected by the water supply of the weeds before sample taking, by the amount of

precipitation before sample taking, the development level of the weeds and the density of their population.

- The primary material of samples (fresh, dried and ground) also affected allelopathy.
- Germination temperature also influenced the end result of biotests.

The effects of extracts prepared from fresh plant parts for test crops can be summarized in the following way:

Italian cocklebur extracts inhibited the germination of sugar beet test crops in a smaller or greater extent; however, factors mentioned earlier affected the power of the inhibitory effect and the rate of effect loss. In general, it can be concluded that the effect of sprout extracts was greater than that of root extracts. In 2003 the germination inhibiting effect of young plant extracts was stronger than that of blooming plants, whereas such difference could not be detected in 2004. As a result of heavier precipitation, extracts had a greater germination inhibiting effect in both years. Out of the two years, this effect of precipitation was less significant in 2004, in which year the weather was more favourable and received more balanced precipitation.

As long as extracts were effective, they affected the growth of the test crops positively, but in some cases inhibitory effects also emerged (e.g. we did not experience the stimulus of samples from blooming cocklebur collected before precipitation if we collected them following precipitation, but we experienced inhibitory effects in several cases). Extracts were more effective on the root growth of the crops than on their sprout growth.

In the case of cress, only some extracts of great concentration could cause the large-scale inhibition of germination. However, their effects on growth varied on a large scale in relation to the antecedents and circumstances of

extract preparation. Regarding the crop parts used for extract preparation we experienced that sprout extracts were mostly of greater inhibitory effect than root extracts. In sprout extracts, the inhibitory effects exercised on test crops were greater before precipitation than after it, when in several cases inhibition did not take place or the extracts had stimulating effects.

In root extracts this phenomenon could not be observed; moreover, several cases showed exactly its opposite. However, the effect was influenced not only by precipitation, but by other factors as well. The growth of garden cress was inhibited by the extracts of blooming plants more significantly in both years, than those of 4-5 leaf plants. Nevertheless, the effect of cocklebur population density was only significant in the case of some samples in 2004. Extracts affected the sprout growth of test plants less, than their root growth.

Maize was usually less sensitive to the same extracts than the above mentioned two test crops, but precipitation, population density and the phenology of donor plants influenced allelopathy in this case as well. Extracts from cockleburs grown in fields inhibited maize growth solely in the case of root samples from dense populations in July, but after precipitation this effect could not be experienced.

In the case of cress and sugar beet we compared the effects of extracts from fresh crop parts with the effect of extracts from the dried and ground pieces of the samples. In the case of cress the effects of extracts from fresh and dried parts were similar (on day 6. $R=0,709$), but the latter was more effective (*Figure 1.*) However, the composition and solubility of allelochemicals effecting on sugar beet modified in the course of drying and grinding so much that there was no correlation between the effects of

extracts from fresh and dry samples on day 10. in respect of germination $R=0,049$, and root growth $R=-0,262$ (*Figure 2.*).

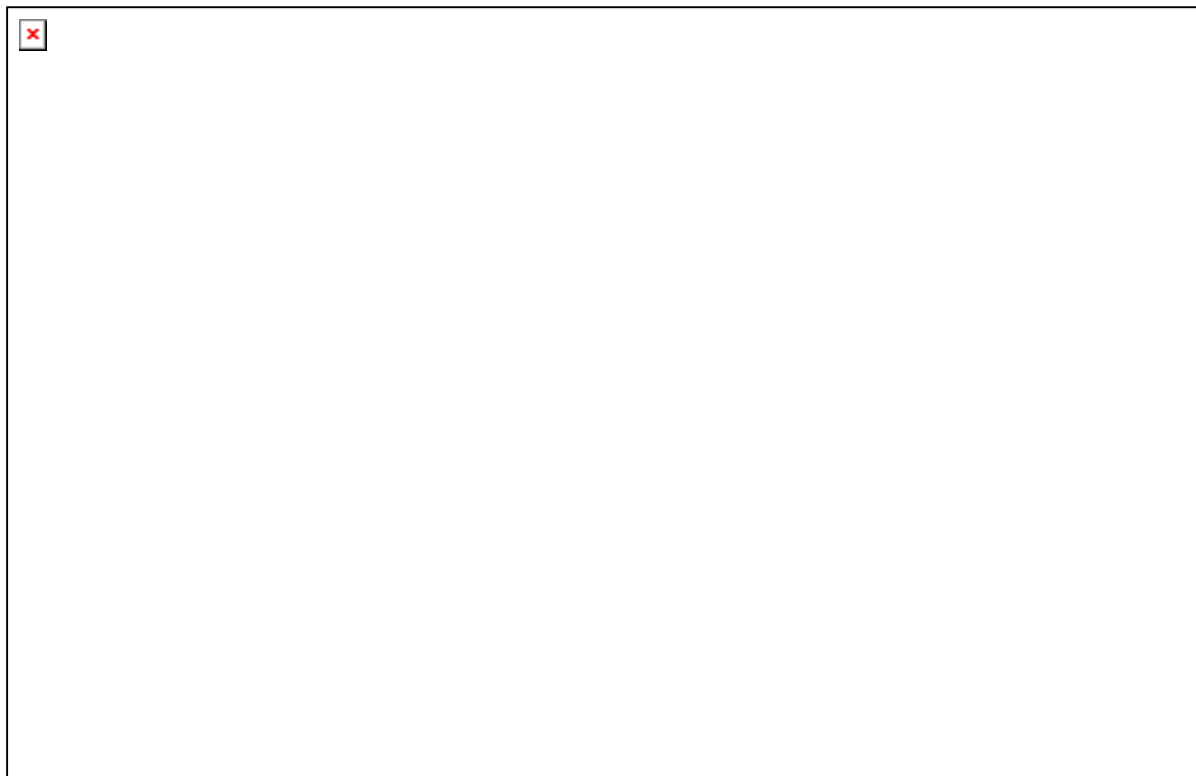


Figure 1. Effects of extracts made from fresh and dried samples of cocklebur on root growth of cress at 6th day

S: shoot extract; R: root extract; T: extract made from thin stand of cocklebur; D: extract made from dense stand of cocklebur.

(Concentration of extract made from fresh cocklebur is 12g/100ml, made from dried cocklebur is equivalent to the fresh cocklebur extract.)

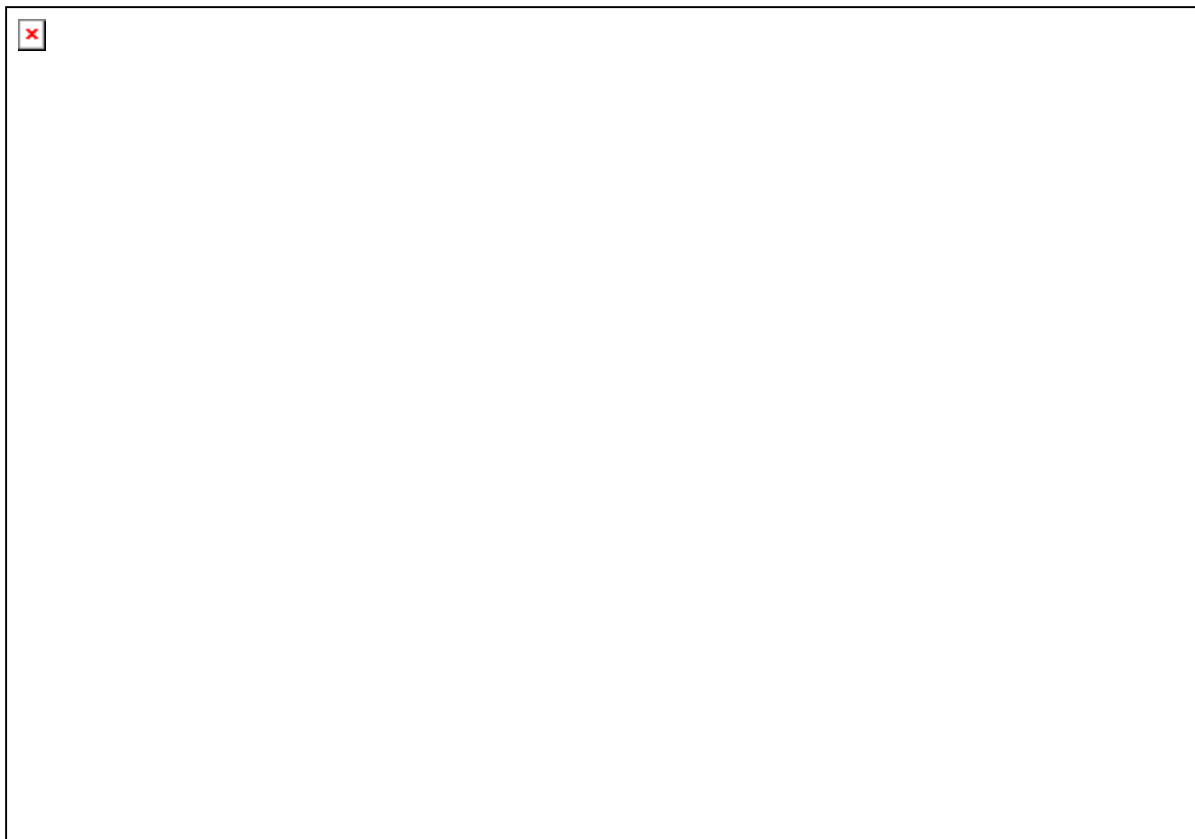


Figure 1. Effects of extracts made from fresh and dried samples of cocklebur on root growth of sugar beet at 10th day

S: shoot extract; R: root extract; T: extract made from thin stand of cocklebur; D: extract made from dense stand of cocklebur.

(Concentration of extract made from fresh cocklebur is 12g/100ml, made from dried cocklebur is equivalent to the fresh cocklebur extract.)

The effects of cocklebur remnants on test crops were determined by the temperature of germination. Inhibitory effects on test crops were more significant at a lower temperature, which was less favourable for germination. The growth of cress was inhibited by extracts in a greater extent at a lower temperature than at room temperature.

In fact, extracts could not inhibit the germination of sugar beet at room temperature, but at 8-10 °C it was inhibited by each extract containing root remnants. However, there was no significant difference in the stimulating effect on sugar beet growth, depending on the temperature of germination. Maize proved to be sensitive to extracts containing sprout remnants at

temperatures unfavourable for germination; however, at room temperature all extracts were ineffective for this test crop.

In the sprouts and roots of cockleburs growing under natural field conditions, as a result of the changeability of allelopathy, we could follow-up the quantitative changes of four allelochemicals: that of trans-cinnamic acid, coumarin, p-coumaric acid and chlorogenic acid. The concentration of the given compounds showed a modification of 4.6-15.5 times in sprouts, and that of 2.6-29.8 times in roots in the vegetation period (*Figures 3., 4.*).

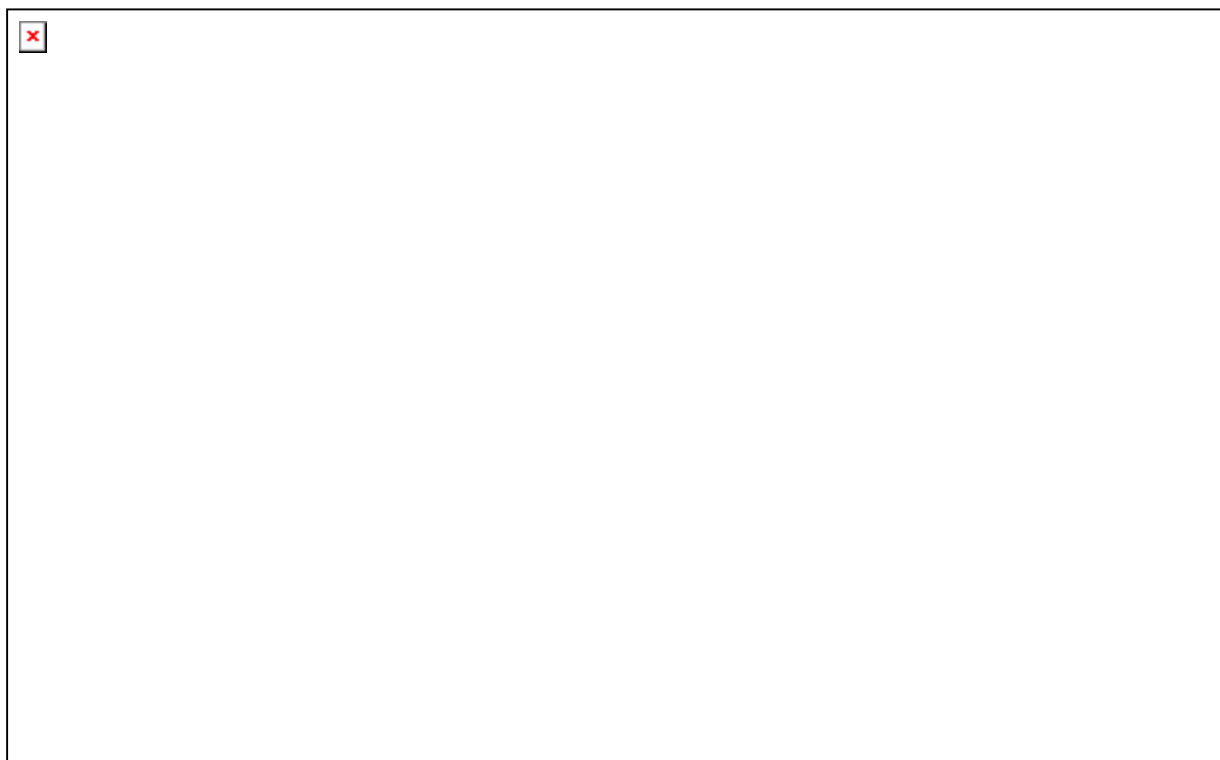


Figure 3.: Changes in amounts of chlorogenic acids and trans-cinnamic acid in shoots and roots of cocklebur

T: extract made from thin stand of cocklebur; D: extract made from dense stand of cocklebur

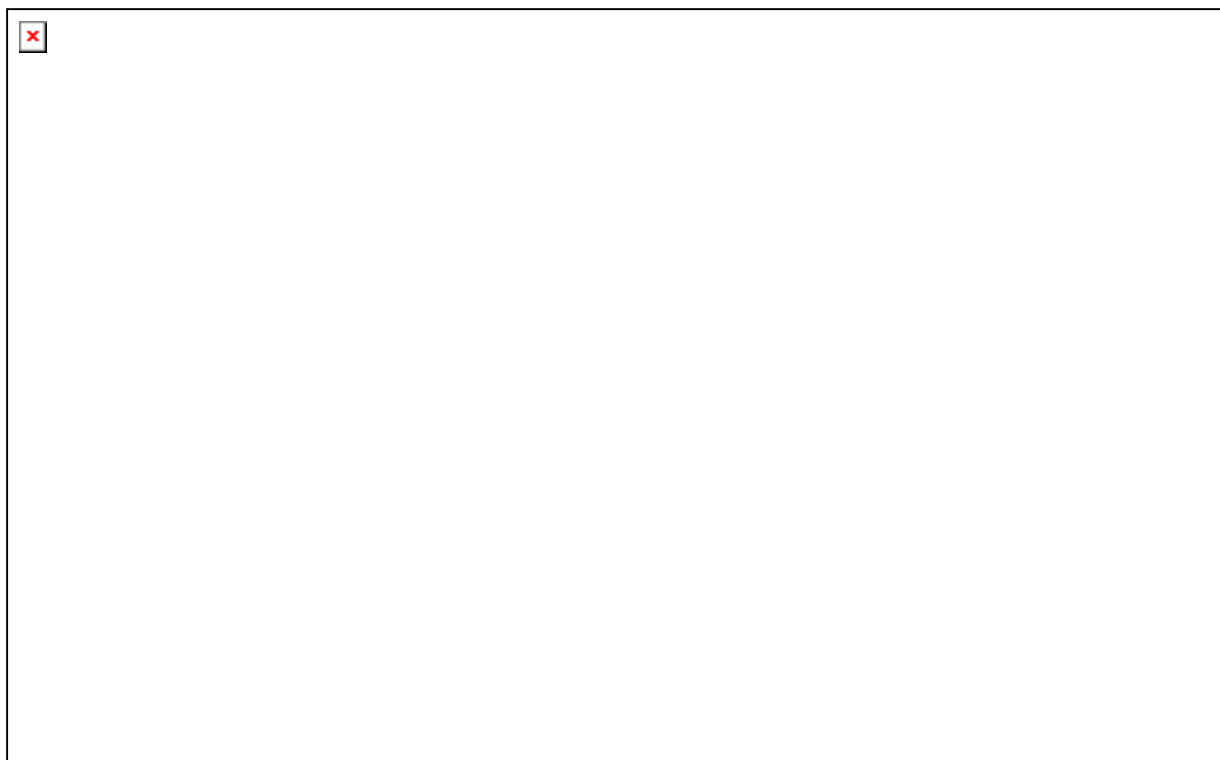


Figure 4.: Changes in amounts of coumarin and p-coumaric acid in shoots and roots of cocklebur

T: extract made from thin stand of cocklebur; D: extract made from dense stand of cocklebur

Although allelopathy is mostly influenced by the joint effects of several compounds, the quantitative changes of p-coumarin acid and trans-cinnamic acid showed a correlation with the effects of extracts on cress. The correlation between the quantity of p-coumaric acid and the early sprout growth of cress was: $y = -7,0535 + (-3,3198 \cdot \ln x)$ ($R^2 = 0,811$) (*Figure 5.*); the correlation with root growth was: $y = -11,009 + (-5,7628 \cdot \ln x)$ ($R^2 = 0,775$) (*Figure 6.*).

The quantitative changes of cinnamic acid showed a correlation with the root growth of cress, which was experienced at a later measurement ($R = -0,743$). However, in other cases we could not draw conclusions from the quantitative changes of any compounds on the growth and germinating power of the test crops, which denoted that the effects of other allelochemicals in cockleburs might be significant.



Figure 5.: Correlation between amounts of p-coumaric acid in shoots of cocklebur and shoot growth of cress treated with extracts of cocklebur at 3rd day.

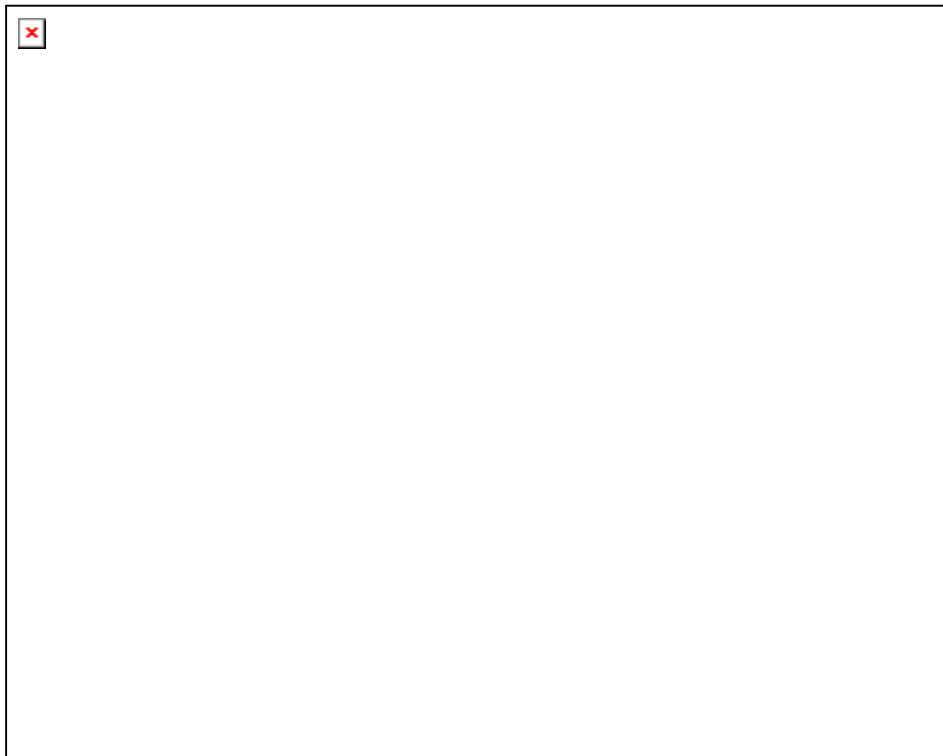


Figure 6.: Correlation between amounts of p-coumaric acid in shoots of cocklebur and root growth of cress treated with extracts of cocklebur at 3rd day.

The changeability of allelopathy caused by cockleburs, which can be experienced on test crops and the significant quantitative differences of allelochemicals depending on external and internal factors make it necessary to find out the factors which are responsible for the changes, to quantify their effects and to take them into consideration in studying allelopathy so that we can use research findings more extensively.

On the basis of our findings, the roles of the studied and other factors can be significant in many cases. Further investigations are needed to find out the effective factors in certain cases of allelopathy and to identify their exact effects. The factors which mostly influence research findings are to be identified, e.g. from the quantity and intensity of precipitation before sample taking, we can only draw direct conclusions concerning the extraction of allelochemicals from crop sprouts. Crop water supply and its changes are caused, besides the effects of occasional precipitation, by the water supply capacity of soil before and after rain, atmospheric dryness etc., so considering these facts, the effect of precipitation can only be considered indirect. In these cases, the measurement of qualities which reflect crop water supply better, such as water potential could be suggested for further examinations.

Results related to studies on *Puccinia xanthii*

One of the potential means of biological defence against cockleburs is *Puccinia xanthii*, which occurred in Hungary in the past years. Our investigations surveyed the infection caused by rust in certain cocklebur populations and the occasional emergence of *Puccinia xanthii* on sunflower hybrids and ragweed.

The infection caused spectacular symptoms (a great number of telio-pustules) on cockleburs by the end of the vegetation period, but as a result of its regular late occurrence (July-August), it could not cause significant damages in the weed population. We can suppose that the lack of early infection was caused by the shortage of inoculum, which can be the consequence of the fact that temporarily, *Puccinia xanthii* cannot live through the winter in Hungary. Therefore, the inoculum arriving from Southern Europe could have started the infections. As a result, its weed regulating effect could not be effective and will not succeed unless earlier infection takes place.

The rust, which we isolated, could not damage the studied sunflower hybrids and ragweed plants in either year.

New and novel results of research

- By biotests we have identified the allelopathy of Italian cocklebur and their remnants for cress, sugar beet and spring barley, as test crops.
- We have found out the influence of the donor plant development on allelopathy.
- Out of external factors affecting donor plants, we have identified the influences of water supply, precipitation conditions, population density, light (artificial and natural, long and short days) on allelopathy.
- Out of direct biotest conditions we have identified the influences of sample preparation, solvent type and germination temperature.
- We have investigated the quantitative changes in the four allelochemicals (chlorogenic acid, trans-cinnamic acid, coumarin and p-coumaric acid) in Italian cocklebur and their correlations with allelopathy.
- We have studied the competition of cocklebur with sugar beet and maize in different years, in early and late emerging cocklebur. We have provided information on the survival and production abilities of late emerging cocklebur in the given cultures.
- We have described the Hungarian occurrence of *Puccinia xanthii*, we have collected data on the rate and type of infection on cocklebur. We have studied the occurrence of the disease on sunflower hybrids and ragweed.

Practical use of findings

The expansion of cocklebur species has accelerated in Hungary in recent years, therefore cockleburs cause severe problems in considerable areas. Despite this fact, there is little information on their compatibility, the factors influencing compatibility, their yield reducing effects in Hungarian row crops, which are mostly endangered.

Considering the late emergence of cockleburs and the good compatibility of late emerging weeds, the paper has provided information on their damaging ability, both in early and late emerging weeds. We have supplemented the investigations with data on the different weather conditions of years, and we have studied a wide range of weed density. Thus we could identify the threshold value of damage in the larger cultures endangered by cocklebur species.

Allelopathy is one of the factors influencing the competitiveness of cockleburs, though its significance is not yet clear. It is of high importance to find out the real role of this phenomenon in the competition of not only cockleburs with cultivated crops, but also in the competition of other weeds with cultivated crops. For this, we have to consider the fact that this form of relationship between crops and other organisms is influenced by several factors. In my paper I have studied factors (e.g. plant development stage, weather conditions etc.), which need further consideration and investigation for the description of allelopathy. In this way we can use our information on allelopathy more effectively.

In the protection against weeds and other pests the minimalization of environmental stress is highly significant for every expert in plant protection. In relation to the regulation of cockleburs, a great number of

living organisms were studied, including *Puccinia xanthii* in Hungary. In the case of *Puccinia xanthii*, just like in all organisms which can be used in biological defence, the potentials for its use and the potential harmful effects of the pathogen are to be found out. The paper presents some further information on this.

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