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Action of Volatile Substances Liberated from Couch Grass

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Abstract. A study was made of the action of volatile substances liberated from the rhizome of couch grass on wheat seedlings. The experiments were made in a closed atmosphere in glass vessels with continual removal of expired CO_2 and the addition of O_2.

Volatile substances liberated from couch grass produced numerous morphological and physiological changes in wheat seedlings. They caused retarding of the growth of overground parts and roots, the excessive formation of root hairs, curling of the roots and bending of the coleoptiles. The respiratory rate was higher in the leaves and roots of the experimental wheat seedlings than in the controls. The changes observed were similar to those produced by hydrocarbons of the ethylene series, which are well documented in the literature.

The allelopathic action of couch grass has been confirmed in much data obtained by laboratory work and from experiments in natural soil conditions (OSVALD 1950, TREIBS 1947, 1959, LAŠTŮVKA 1955, 1960, 1961, 1964, KOMMEDAHL, KOTHEIMER, BERNARDINI 1959, WELBANK 1963, PLHÁK 1964). Some authors considered volatile essential oils, isolated from the rhizome, to be the cause of the allelopathic properties (TREIBS 1947, 1959, LAŠTŮVKA 1960,1961). Other authors thought these properties to be due to non-specific substances forming and being liberated during decomposition processes of the underground organs (WELBANK 1963). The first theory has a certain amount of uncertainty, since sufficient evidence has not yet been provided of the liberation of these volatile substances from the growing organs of couch grass or of the biological action of such substances under natural conditions.

Materials and Methods

The experiments were made with couch grass (*Elytrigia repens* (L.,) DESV. = *Agropyron repens* L.) and the spring wheat "Niva" in a greenhouse at a temperature of 15° ± 3° C.

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Healthy couch grass rhizomes were cut into sections about 12 cm. long, moistened in Knop's nutrient solution and placed in amounts of 200 g in experimental vessels composed of the lower parts of exiccators covered with a bell glass. The total volume of the vessel was about 3 litres. They were left for 10 days and when the rhizomes started to grow (and produce roots), Petri dishes with wheat seeds were placed over the rhizomes. The Petri dishes contained three sheets of filter paper moistened with water. A dish containing 200 ml KOH was placed on the floor of the experimental vessel to absorb

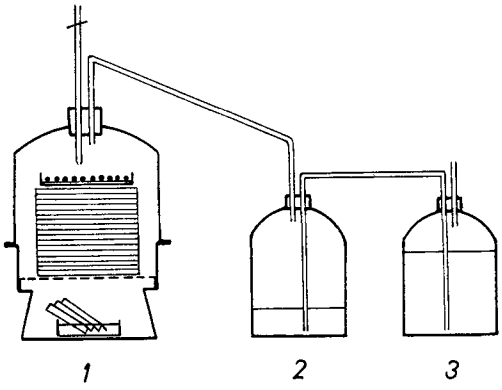

Fig. 1. Diagram of experimental set-up. Vessel 1 contains dish with KOH solution, couch grass rhizomes and Petri dishes with seedlings. Vessel 2 contains oxygen and water, vessel 3 contains water. System of vessels connected so that water flowing from vessel 3 forces oxygen into vessel 1 at rate comparable to oxygen consumption of plant tissues.

the CO_2 produced. Folded filter paper was placed in the KOH solution in order to increase the absorbing area.

During the experiment the vessels were kept airtight. The oxygen consumed was replaced from a bottle at the side connected to the bell glass with rubber tubing. In order to ensure that there was an adequate amount of oxygen above the couch grass, oxygen was forced into the experimental vessel under slight pressure by raising the water level in a further bottle connected with the oxygen bottle by a rubber tube (see diagram in Fig. 1).

The wheat seedlings were kept moist with water during germination by

Table 1. Growth of wheat seedlings placed alone and together with couch grass in experimental vessels for 10 days

Age of plants in days	Length of plants organ in mm				Values t_{17}	
	Control plants		Plants with couch grass			
	Roots	Leaves	Roots	Leaves	Roots	Leaves
5	49·4	—	29·5	—	—	—
7	80·6	—	41·0	—	—	—
10	107·3	110·7	66·2	67·4	6·71**	8·30**

** highly statistically significant difference (P = 0.01)

tubes placed in the neck of the bell glass. The control wheat-seedlings were kept under the same conditions, but without the presence of the couch grass in the experimental vessel.

During 10 days of germination the seedlings were photographed and the lengths of the primary roots measured. After 10 days, at the end of the experiment, the lengths of the leaves were also measured and the respiratory rate of the leaves and roots was determined manometrically by the Warburg apparatus. Differences in growth and respiration were evaluated by the t-test. The experiments were repeated three times in succession.

Table 2. Respiration rate of roots and leaves of wheat seedlings placed alone and together with couch grass in experimental vessels. Age of plants 10 days. Respiration rate expressed as O_2 consumption per gram. tissue dry weight per 60 min.

$\mu l\ O_2$				Values t_4	
Control plants		Plants with couch grass			
Roots	Leaves	Roots	Leaves	Roots	Leaves
3221	3629	4441	3991	5.05**	3·1*

* significant differences (P = 0·05)
** highly significant differences (P = 0·01)

Results

Figs. 2—5 and Tab. 1 show the growth of wheat seedlings placed over couch grass and in control vessels during 10 days.

A number of changes were observed in the wheat seedlings placed in an enclosed atmosphere with vegetating couch grass rhizomes in comparison with the controls. The growth of the overground parts and roots of the experimental plants was greatly inhibited. Inhibition of the growth of the primary roots was visible from the fourth day of germination. This inhibition of growth became more marked in the next few days and then became less (Figs. 2, 4 and 5). At the end of 10-day's growth, the roots of the wheat seedlings grown over couch grass rhizome were 38·4% shorter than those of the controls. The length of the first leaf of the experimental plants was also 38% less than that of the control plants.

Other morphological and physiological changes were noted in the experimental seedlings, in addition to inhibition of growth. Swelling of the coleorhiza was visible at the beginning of germination, later there was excessive growth of root hairs. The growing roots became curved and coiled particularly at the beginning of growth (Figs. 2 and 3). This was probably connected with a disturbance in the negative geotropic reaction of the roots. Similar disturbances in growth correlations — bending of coleptiles — were seen also in the overground parts (Fig. 5). The respiration rate was significantly higher in the overground and root parts of the experimental plants than in the controls (Tab. 2).

Discussion

The results of the experiments showed that vegetating couch grass rhizomes have a definite action in wheat seedlings when placed together in an enclosed

space. Since oxygen deficiency and excess CO_2 in the experimental vessels resulting from the metabolic activity of the plant tissue were excluded, it can be assumed that the changes were produced by volatile substances liberated from the tissues of the couch grass.

The same changes were observed in all three repetitions of the experiment. The character of the reactions can be compared in some ways with the reaction of plant organs to the presence of biologically active substances of ethylene type. The most important physiological changes produced by ethylene and also to a varying extent in the present experiments, were as follows: Inhibition of growth in length observed on wheat seedlings (MACK and LIVINGSTON 1933), retardation of root growth (BORRIS 1943), loss of negative geotropic reaction of stems (NELJUBOV 1911, DOSTÁL 1942) and loss of positive geotropic reaction of roots (MOLISCH 1937, ZIMMERMAN and HITHCOCK 1933), inhibition of growth in length and thickening of stems and roots and excessive root hair formation, etc. (EVENARI, 1961). Other authors reported increased tissue respiration as a characteristic reaction to ethylene (HUELIN and BARKER 1939, HERRERO and HALL 1960), a finding also made in our experiments. Since the production of active substances of the ethylene type has been demonstrated in many plant tissues, including fruits, leaves, flowers, stems, tubers and roots of various plants (MOLISCH 1937, EVENARI 1961), also including the leaves and roots of couch grass (PORUTSKY, LUCHKO, MATKOVSKY 1962), it can be assumed that the changes observed in our experiments were at least partly due to the action of substances of this type.

The biological activity of volatile substances from the rhizome of couch grass was also investigated by LAŠTŮVKA (1961, 1964) and MINÁŘ (1961). These authors worked with substances obtained by extracting ground couch grass rhizomes in ether. The volatile fraction of this extract caused big changes in many biological objects, when acting in an enclosed space during short time. After longer time cell, tissue and organ necrosis occurred. The authors explained the action of volatile substances in the extract by the presence of the unsaturated hydrocarbon, agropyrene, which was isolated from the rhizome of couch grass by TREIBS (1947, 1959).

Since agropyrene is an unsaturated hydrocarbon similar to the biologically active hydrocarbons of the ethylene series, it could have a similar biological action. The reports of the above authors, however, contain no mention of changes similar in character to those found in our experiments. These differences may be due to the big differences in the concentration of the substances used in both types of experiments. But qualitative differences in the effective substances in both experiments cannot be excluded in view of the different manner in which they were attained.

In spite of the certain specificity of the reactions of the experimental plants on the active substances liberated from couch grass, found in our experiments, no definite conclusion can be drawn about their nature without closer identification. Our results, however, confirm allelopathic properties of couch grass conditioned by the liberation of biologically active volatile substances from vegetating rhizomes into the environment.

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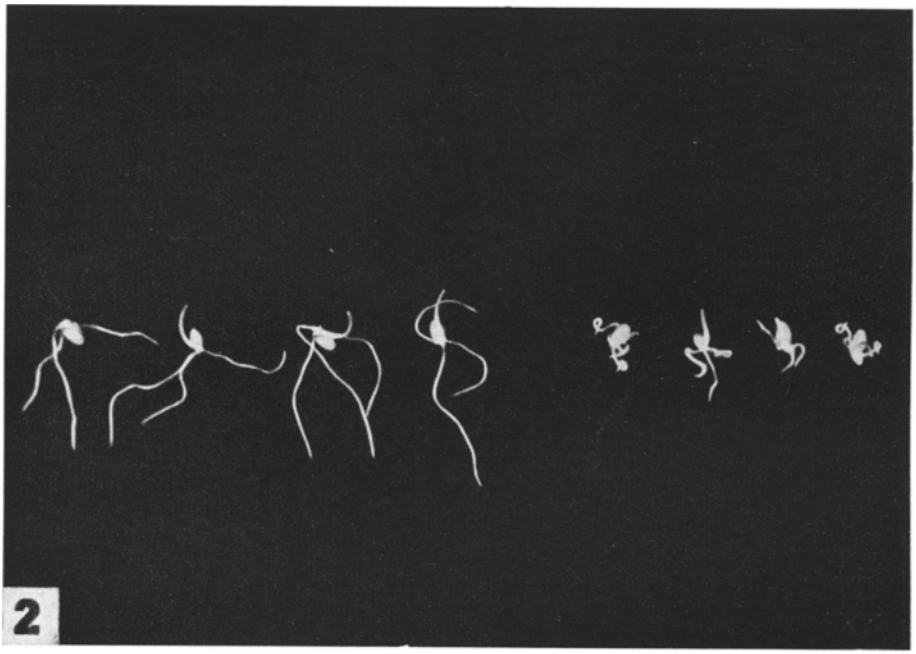
Sledovali jsme účinek volatilních substancí uvolňujících se z vegetujících oddenků pýru na klíční rostlinky pšenice. Pokusy byly prováděny v uzavřené atmosféře ve skleněných nádobách při permanentním jímání vydýchaného CO_2 a doplňování O_2. Pokusné nádoby byly umístěny ve skleníku.

Volatilní substance pýru vyvolávaly četné morfologické a fyziologické změny klíčních rostlin pšenice. Docházelo k inhibici růstu nadzemních částí i kořínků, vytváření přebytku koře-



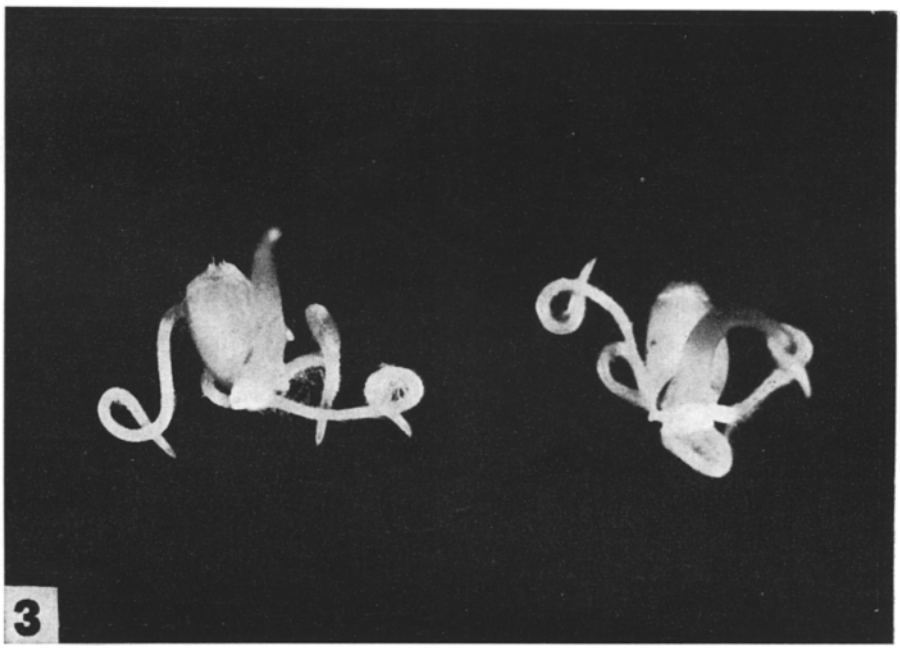
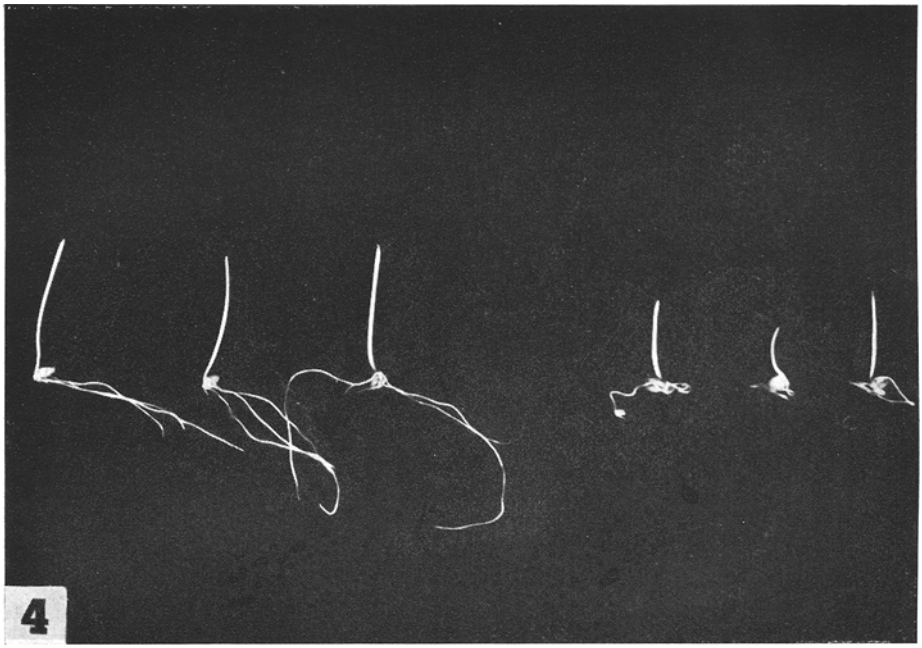


Fig. 2—5. Growth of wheat seedlings placed in experimental vessels without couch grass (control — on left) and with couch grass — on right: Fig. 2 — after 6 days, fig. 3 — after 6 days (only plants growing with couch grass — detail), fig. 4 — after 8 days and fig. 5 — after 9 days growth.



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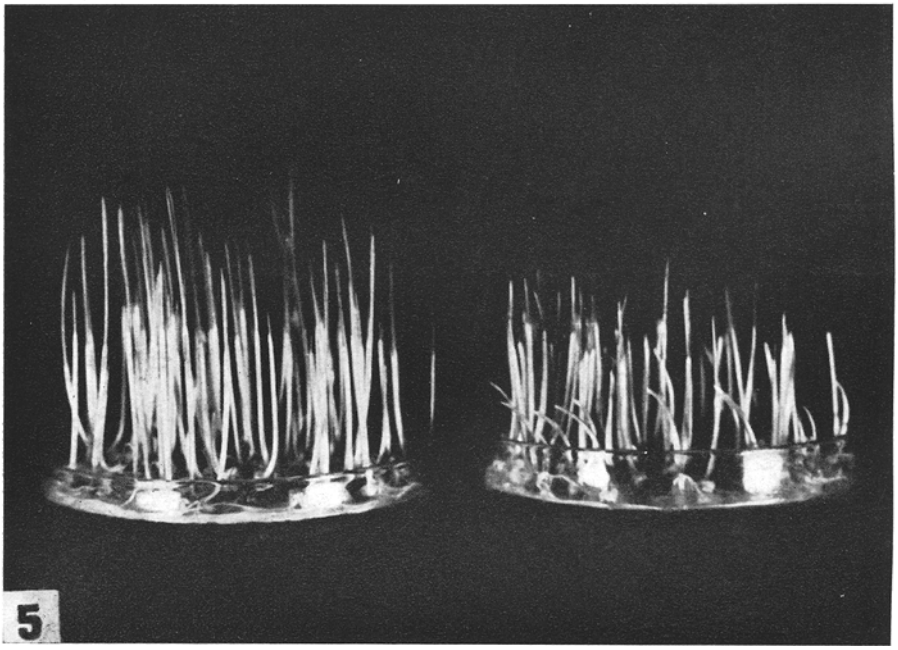

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nového vlášení, zakrucování kořínků a ohýbání koleoptilí. Intenzita respirace listů i kořenů pokusných rostlin pšenice byla proti kontrole patrně zvýšena. Pozorované změny byly podobné v literatuře hojně popisovaným změnám, které vyvolávají uhlovodíky etylénové řady.

Ф. Плгак, И. Гелан, Кафедра физиологии растений и генетики, факультет естествознания, Университет им. Я. Э. Пуркинье, Брно: **Изучение влияния летучих веществ пырея.** — Biol. Plant. **7** : 368—373, 1965.

Мы изучали влияние летучих веществ, освобождающихся из вегетирующих корневищ пырея, на прорастающие растения пшеницы. Опыты проводились в закрытой атмосфере в стеклянных сосудах при постоянном поглощении дыханием освобожденного CO_2 и дополнении O_2. Опытные сосуды были помещены в теплице. Летучие вещества пырея вызывали многие морфологические и физиологические изменения прорастающих растений пшеницы. Было вызвано торможение роста надземных частей и корешков, образование избытка корневых волосков, закруживание корней и изгибы колеоптилей. Интенсивность респирации листьев и корней опытных растений пшеницы по сравнению с контролем очевидно повышена. Наблюдаемые изменения похожи на часто описываемые в литературе изменения, вызываемые углеводородами этиленового ряда.