Short Communication

DECREASED CYTKININ PRODUCTION IN THE ROOTS AS A FACTOR IN SHOOT SENESCENCE

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Summary. The cytokinin content of root exudate of sunflowers increases during the exponential growth phase of the plants. The concentration of cytokinins drops, however, by a factor of ten when the plants have reached their final size. The reduced supply of cytokinins from the root to the shoot is regarded as one of the factors bringing about shoot senescence.

It has been shown recently that the roots of sunflower plants are sites of cytokinin synthesis and that three such factors are translocated to the shoot through the transpiration stream (KENDE, 1964, 1965; WEISS and VAADIA, 1965; KENDE and SITTON, in press). Cytokinins have also been found in root exudate of tobacco (KULAEVA, 1962), of grapes (LOEFFLER and VAN OVERBEEK, 1964), of maple (NITSCH and NITSCH, 1965), and of beans and banana (ITAI and VAADIA, unpublished data). These findings pose the question as to physiological significance of these hormones.

Removal of the root causes decrease in protein content and enhances senescence in the leaves (CHIBNALL, 1939; MOTHERS, 1960; Parther, 1964) and either of these processes is delayed by application of a cytokinin (RICHMOND and LANG, 1957). It appears possible that, in the intact plant, cytokinins formed in the root and translocated with the transpiration stream to the shoot have a similar effect, in other words, that they function in the endogenous regulation of protein metabolism in the leaves. This assumption is supported by two lines of evidence. KULAEVA (1962), using an indirect approach, concluded that attached leaves contained optimal amounts of root-supplied cytokinins. Removal of the roots caused a deficiency in these factors and rendered the leaves sensitive to applied kinetin (6-furfurylaminopurine). More direct evidence has been supplied by experiments of SHAH and LOOMIS (1965), and ITAI and VAADIA (1965). SHAH and LOOMIS found a decline in the RNA and
protein content of sugar beet leaves if the plants were subjected to water stress. These symptoms of senescence were prevented by spraying the plants with benzyladenine (6-benzylaminopurine), a synthetic cytokinin. Itai and Vaaadia reported that the cytokinin concentration of the root exudate from sunflower plants was markedly reduced after a 24-hour period of water stress. Thus, it appears that premature senescence of leaves can be initiated not only by removal of the root but also by subjecting the latter to unfavorable conditions. Water stress very likely causes leaf senescence by reducing the production of cytokinins in the roots and, consequently, reducing the cytokinin supply to the shoot.

At this point, it seemed important to determine the cytokinin content of root exudate of plants during their development, and in particular to investigate whether changes could be observed as the plants underwent natural aging. Such experiments were carried out with sunflower plants.

Sunflowers (*Helianthus annuus* L., local variety) were raised in water culture on half-strength Hoagland solution in the greenhouse. Groups of hundred plants were topped at different stages of development and root exudate was collected for 24 hours as described earlier (Kende, 1964). The exudate was frozen, lyophilized, and the dry matter taken up in 80% ethanol. This solution was centrifuged and the supernatant paper-chromatographed using n-butanol-acetic acid-water (4:1:1, v/v) as solvent (Itai and Vaaadia, 1965). The region corresponding to Rf 0.5—0.8, which contains the factor(s) with high cytokinin activity, was eluted with 80% ethanol. The dried eluates were taken up in 2 ml of water and bio-assayed by means of the soybean callus test (Miller, 1965).

Height of the plants, total number of leaves, number of wilting leaves, and stage of floral development were recorded at intervals of 5—7 days. For determining floral development, shoot apices were dissected and examined under the microscope. Floral development was expressed in the following units: Stage zero = vegetative apex; Stage 1 = apex with first signs of transition to the reproductive phase; Stage 2 = diameter of the inflorescence bud 4—12 mm; Stage 3 = 13—20 mm; Stage 4 = 21—35 mm; Stage 5 = 36—50 mm; Stage 6 = open inflorescence.

The observations on growth and development are summarized in Table 1. The total amount as well as the concentration of cytokinin(s) in the root exudate increased during the exponential growth phase of the plants but dropped by a factor of ten at the onset of senescence, when plants had reached their full size (Table 2).

In the roots of sunflower plants, cytokinin synthesis appears to be confined to the root apex (Weiss and Vaaadia, 1965). This agrees with the hypothesis that cytokinins are produced in growing, meristematic root tissue (Goldacre, 1959). As the plant is growing, more secondary roots are formed, resulting in a steady increase of sites of cytokinin synthesis. This is reflected in the rising cytokinin level of the root exudate during the exponential growth phase (Table 2). Brouwer (1962), working with peas and tomatoes, observed a close correlation of shoot and