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CYTOKININ LEVEL AND SENESCENCE OF LEAVES IN SYCAMORE SEEDLINGS

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The cytokinin level in root exudate of sycamore seedlings during senescence decreased by a factor of 7 as compared to non-senescing seedlings. The decrease in new root growth and a concomitant decrease in xylem sap cytokinin level is considered to be a major factor leading to leaf senescence. The seedlings grown in the greenhouse showed evidence of senescence after 4-5 months under a constant temperature and photoperiod regime. Although a decrease in new root growth and xylem sap cytokinin level was correlated with leaf senescence, the environmental factor(s) responsible for these changes remain unknown.

INTRODUCTION

It has been shown recently that cytokinins (or cytokinin-like substances in sycamore (*Platanus occidentalis* L.) seedlings are translocated from roots to shoots through the transpiration stream (Dhillon, 1975). Cytokinins have also been reported in xylem exudate of other plants including tree species like *Acer pseudoplatanus* (Horgan *et al.*, 1973); *Pseudotsuga menziesii* (Morris *et al.*, 1976); and *Acer saccharum* (Dumbroff & Brown, 1976). The above findings raise the question as to the physiological significance of these root hormones.

Richmond and Lang (1957) showed that kinetin retarded senescence of detached xanthium leaves in that it caused a delay in net breakdown of protein and chlorophyll. These results have been verified by a number of workers. In intact plants, cytokinin-like substances produced by roots may also regulate the senescence of leaves (Kulaeva, 1962; Kende & Sitton, 1967; Wareing & Seth, 1967; Fletcher, 1969; Adedipe *et al.*, 1971). Thus, it appears that in the intact plants, cytokinins formed in the root are translocated with the transpiration stream to the shoot, and function in the endogenous regulation of protein metabolism in the leaves (Kulaeva, 1962; Shah & Loomis, 1965; Itai & Vaadia, 1965). In addition, removal of roots results in a decrease in protein content and enhances leaf senescence (Chibnall, 1939; Mothes, 1960; Parthier, 1964).

The conditions unfavourable for root growth very likely cause disruption in the physiological processes of leaves by reducing cytokinin synthesis in the roots.

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For example, increased soil moisture stress (Itai & Vaadia, 1965), root temperature (Skene & Kerridge, 1967), osmotic stress (salinity) (Itai *et al.*, 1968), and flooding (Burrows & Carr, 1969; Vasey, 1970), all have been shown to cause a decrease in cytokinin concentration in the xylem sap of various plant species. A normal seasonal change in trees (Dumbroff & Brown, 1976) as well as natural aging in annual plants (Sitton *et al.*, 1967; Tal *et al.*, 1970) have also been shown to cause a change in cytokinin level, which appears independent of the fluctuation caused by perturbations in environmental conditions. The present study was undertaken to determine, if in the absence of natural aging as in annual plants and seasonal activity as in trees, the cytokinin level, new root growth, and leaf senescence were a related phenomenon in sycamore seedlings.

Materials and Methods

Sycamore (*Platanus occidentalis* L.) seedlings were raised in water culture on half-strength Hoagland solution 2 (Hoagland & Arnon, 1950) in the greenhouse under constant temperature (24±4°C) and photoperiod (16-hour). Thirty-two seedlings were harvested at different ages (119, 133 and 144 days) and root exudate was collected making a total of 96 seedlings. Since root pressure was not sufficient to provide enough xylem sap, it was necessary to physically force the sap out of the stems. This was accomplished by using a pressure chamber. The plant stem was cut about 4 cm above the root collar and the root system placed in the pressure chamber with the cut surface extending outside. Tissue external to the cambium was peeled back so that only xylem tissue was exposed. A glass capillary tube was attached to the exposed stem by a short piece of rubber tubing and a collection flask placed under the other end of the tube. Pressure in the chamber was run at 300 psi and exudate collected over a 10 min period from each root system. Any pressure between 250 and 500 psi, may be used, but collection time should not exceed 10 minutes in order to avoid progressive contamination of the xylem sap with liquid from other sources within the plant (Vasey, 1970).

After collection of the exudate, an equal volume of 95% ethanol was added in order to restrict the development of bacteria and fungi in the exudate. The mixture was then evaporated to dryness under vacuum at 35°C using a flash evaporator. The dry residue was dissolved in 95% ethanol and the liquid streaked on Whatman 3 chromatography paper. Descending chromatograms were developed over a distance of 30 cm with n-butanol : acetic acid : water (4 : 1 : 1, V/V/V). The developed chromatograph was allowed to dry at room temperature and then divided into 10 parts representing the desired Rf values. The region corresponding to Rf 0.2–0.9 was eluted with 95% ethanol. Rf 0.1 and 1.0 appeared inhibitory to soybean callus bioassay and were not included. The levels of kinetin-like activity were determined by soybean callus-growth bioassays (Miller, 1963). The cytokinin level in the original exudate samples was calculated by using the standard curves. Two peaks of activity (Rf 0.3, 0.7) reported for sycamore (Dhillon, 1975) are similar to those reported for other plant species

(Kende, 1964; Weiss & Vaadia, 1965; Skene & Kerridge, 1967; Skene, 1968, 1970; Vasey, 1970).

Height of seedlings, number of leaves, number of yellow or dry senescing leaves, and new root growth were recorded at three age groups starting before leaf senescence began and following with increase in number of senescing leaves.

Results

The total amount as well as concentration of cytokinins in root exudate was maximum during the active growth of seedlings (119 day age group) when most of the seedlings (30 out of 32) were showing active root growth as indicated by white root tips (Table I). During the next 14 days (i.e. 119 to 133), the seedlings had grown 1.5 fold in height and number of leaves but the onset of senescence was apparent from the appearance of yellow leaves. The onset of senescence was accompanied by a decrease in both total amount and concentration of cytokinins in root exudate. Also the number of seedlings showing new root growth decreased from 30 to 21. When seedlings were allowed to grow for 144 days until 1/4th of the leaves turned yellow or dried, the total amount as well as concentration of cytokinins in root exudate showed a seven-fold decrease as compared to actively growing (119 day old) non-senescing seedlings. None of the 144 day old senescing seedlings appeared to show white root tips.

Discussion

In the roots of sycamore seedlings, cytokinin synthesis appears to be confined to the actively growing white root tips as reflected by the decrease in cytokinin level in root exudate when the number of seedlings showing white root tips decreases. This is in agreement with the results of Weiss and Vaadia (1965) for the roots of sunflower, and supports the generally held hypothesis that cytokinins are produced in growing meristematic root tissue (Goldacre, 1959).

As cited earlier, the role of cytokinins in senescence of detached as well as intact leaves is well established. Therefore, decreased cytokinin level in root exudate (due to decrease in white meristematic root tips known to be sites of cytokinin synthesis) results in reduction of cytokinin supply from roots to the leaves and may be one of the major factors leading to senescence. This hypothesis is supported by the results of the present study as well as other studies on different plant species (Kulaeva, 1962; Kende & Sitton, 1967; Wareing & Seth, 1967; Fletcher, 1969; Adedipe *et al.*, 1971).

Although it is suggested that a decrease in new root growth and xylem sap cytokinin level results in leaf senescence in cycamore, the factor(s) which initiate their decrease remain unknown. The seedlings began senescing after 4–5 months even under uniform greenhouse conditions where temperature and photoperiod regime were constant. In sugar maple, an increase in numbers of white root tips and cytokinin-like substances followed winter chilling requirements (Dumbroff & Brown, 1976). Such studies have not been done with sycamore. In the present study, the lack of chilling as a factor which leads to senescence remains to be

TABLE I

Levels of cytokinins in the root exudate as related to senescence of leaves in sycamore seedlings. Thirty-two seedlings were harvested on every sampling date

Age of seedlings from germination (days)	Height of seedling (cm)	Number of leaves	Total number of yellow or dry leaves	Number of seedlings showing new root growth (white root tips)	Total volume[a] of root exudate (ml)	Total amount of cytokinins in μg kinetin equivalents	Concentration in kinetin equivalents (μg/l)
119	33·61±3·39[b]	21·56±4 49	0	30	102·50	37·60	336 83
133	52·12±5·61	31·81±10·47	24	21	176·25	20·13	114·20
144	56·10±6·68	32·09±7 45	168	0	96·75	5·12	52 92

[a] root exudate collected at 300 psi for 10 minutes

[b] standard deviations

established. Natural aging in annual species at the end of growth cycle (Sitton *et al.*, 1967; Tal *et al.*, 1970) leading to a decrease in the amount of cytokinin is, however, not a factor in sycamore seedlings.

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