Ethylene biosynthesis, ripening and senescence behavior of tobacco (*Nicotiana tabacum* L.) leaves

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Abstract

Ethylene is a plant growth regulator that promotes senescence of plant tissue. Studies regarding the role of ethylene production on Oriental tobacco (*Nicotiana tabacum* L.) leaf ripeness and senescence are limited. The purpose of this study was to investigate the rate of ethylene production by intact, attached leaves of tobacco plants, Oriental type, during ripening and senescence. Plants were grown in the field during the 2001-growing season. Twelve samplings and measurements were carried out every other day throughout the whole period of leaf development, mature and senescence starting 70 days after transplanting (DAT) up to 92 DAT. Ethylene production of leaves declined up to 76 DAT. Senescence of lower leaves began around 78 DAT when leaves began to lose their chlorophyll and a progressive increase of ethylene production appeared. In addition, during this period leaf dry weight began to decrease. Ethylene production is reached in a peak, a climacteric-like surge, within 6 days after the beginning of increase and after the beginning of rapid chlorophyll breakdown and dry matter loss. This peak involved a 5-6 fold increase in the rate of ethylene production. The subsequent rise of ethylene production appears to be associated with the rapid phase of chlorophyll breakdown, and may indicate the final stage of senescence process.

Media summary

The process of ripeness and senescence of Oriental tobacco leaves was investigated in order to evaluate their effects on yield and quality of tobacco.

Key Words

Oriental tobacco, ethylene, chlorophyll, dry matter, ripeness, senescence.

Introduction

Ethylene is a plant growth regulator found in many plants and plant parts. It has long been known to promote fruit ripening and senescence of plant tissue (Miles et al. 1972; Abeles 1973; Mattoo and Suttle 1991). Although previous studies showed a decrease in ethylene production with leaf age (Mattoo and Suttle 1991), Morgan et al. (1992) found that in cotton leaves a peak of ethylene production occurred during their senescence. Ethylene is known to be involved with both chlorophyll degradation in leaf and with the abscission process (Aharoni et al. 1979). Tobacco does not have an abscission zone and its leaves are not normally shed (Woodlief and DeJong 1978).

Weybrew et al. (1984) stated that physiological maturity and ripeness are not the same; maturity is the attainment of maximum leaf dry weight which indicates the transition from growth to senescence, while ripeness occurs about 12 days after maturity. The degree of leaf ripeness at harvest is an important factor determining the suitability of cured leaves for use in smoking products. The color changes are usually the principal guides in judging ripeness (Peedin 1999).

Oriental type tobacco is important for Greek farmers since it brings in significant income and may utilize low fertility soils, which are unproductive for other crops. Until now, studies concerning the process of tobacco leaf ripeness and senescence focused on other types than Oriental. In this study, the changes in the patterns of ethylene production, chlorophyll content and dry weight of leaves in relation to the ripeness and senescence of leaves in Oriental tobacco were investigated.

Methods

A field experiment was established during the 2001 growing season in Serres, North Greece, using Oriental tobacco (basma, variety BZ 7), in order to investigate the ripening process during senescence of leaves on plants.

Tobacco seedlings were transplanted to the field when plant averaged about 12 cm in height. The rows were 0.5 m apart with plants spaced 0.12 m apart in the rows. Plants in field were grown according to normal practices used for Oriental tobacco production (Tobacco Institute of Greece 1996). At 60 DAT (days after transplanting) the 2-3 lowest weak and pale ground leaves of all plants were stripped off which are of no commercial value. Twelve samplings and measurements were carried out every other day throughout the whole period of leaf development, maturity and senescence starting 70 DAT up to 92 DAT at the different row each time. Each row of 60 plants comprised one sampling plot and each sampling plot replicated 4 times. Plots of each replicate were distributed randomly. Sampling was performed by removing 3 fully expanded lower leaves per plant, which had uniform size and mature stage from the accurately same stalk position. Before each sampling the relative amount of chlorophyll content of leaves was determined using a Minolta chlorophyll meter, model SPAD-502. The 3 lower leaves of each plant per plot were measured at both sides and the average of these readings accurately represent leaf greenness for that leaves. The ability to predict chlorophyll content on a leaf area basis from SPAD readings was demonstrated in several crops (Dwyer et al., 1991: Yang et al., 2003).

The harvested leaves from each plot were weighted directly to determine the fresh weight per leaf and then 70-80 leaves weighted again and placed into 3 L darkness jars. The 4 jars were then sealed and were incubated at a constant temperature of 20?C for 3 hours. Then, ethylene production by leaves were determined by withdrawing a 1 ml headspace gas sample from the jars with a syringe, and injecting it into a Varian 3300 gas chromatograph, equipped with a stainless steel column filled with Porapak, length 100cm, diameter 0.32 cm at 50?C and a flame-ionization detector at 120?C. The carrier gas was N_2 at a flow rate of 20 ml min^{-1}. After ethylene measurements the same leaves dried at 70?C for 96 hours, to constant dry weight, in a forced-air oven and reweighed for determining the leaf dry weight.

Means of four replicates were recorded for each of the above measurements. All data were subjected to analysis of variance. Least significant difference (LSD) values were calculated and used for mean separation.

Results

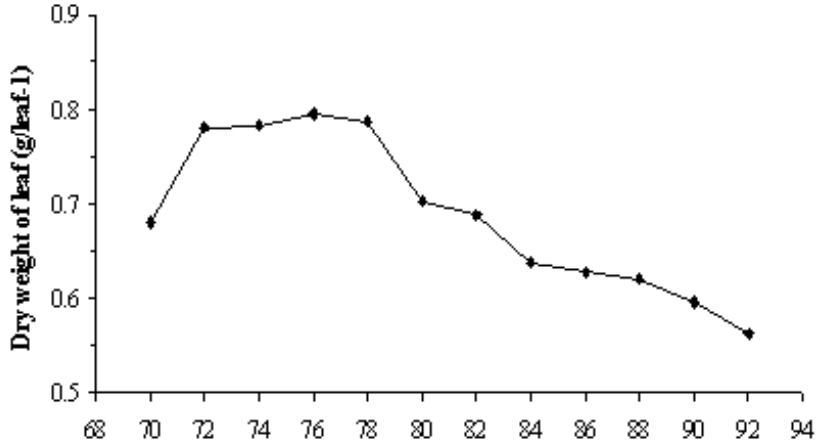
The ripening and senescence behavior of tobacco leaves was studied in terms of leaf dry weight, chlorophyll content and ethylene production in twelve samplings and measurements taken during a period between 70 and 92 DAT. The time course of chances in these parameters among the samplings is shown in Figure 1. Leaf dry weight, chlorophyll content and ethylene production by leaves showed significant fluctuations during the sampling period. The chlorophyll content of leaves, as indicated by SPAD values, decreased slightly between 70 and 76 DAT. During this period ethylene production of leaves declined progressively to a lowest value, while dry weight of leaf after an initial significant increase (70-72 DAT) remained relatively constant. Senescence of lower leaves began around 78 DAT when leaves began to loss their chlorophyll. Beyond day 78 the progress of leaf senescence was reflected by rapid loss in leaf chlorophyll and continuous decrease of leaf dry weight. These changes were associated with a progressive increase of ethylene production to a peak about 5-6 fold over of the lowest value.

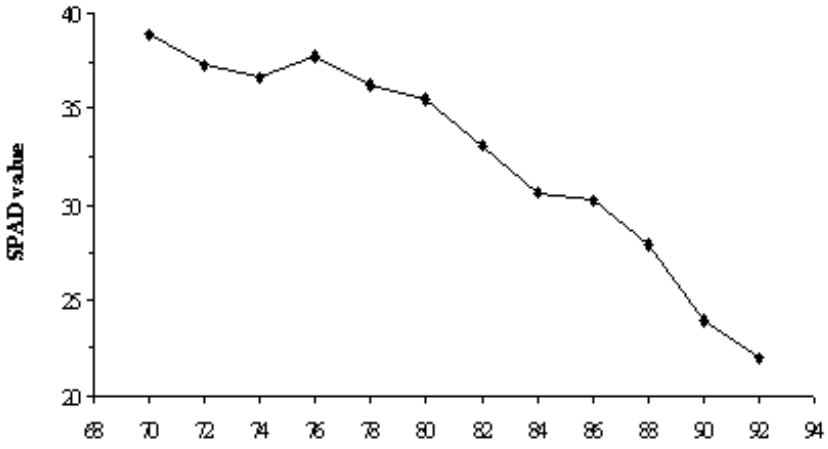
The maximum ethylene production is reached within 6 days after the beginning of increase. It is convenient to term this phenomenon a leaf ethylene climacteric. The period of the most rapid

senescence, as shown by the chlorophyll and dry weight loss from 78 to 84 DAT, coincides with the increase rate of ethylene production on just those days. Moreover, the period of increased ethylene production lasts for around 6 days. Similar results have been reported in the literature for other plant species. An increase rate of ethylene production with increasing senescence has been reported for oat leaves (Gepstein and Thimann 1981) and for cotton leaves (Morgan et al. 1992).

Conclusions

Ethylene seems to be involved in the regulation of senescence of Oriental tobacco leaves. In addition, results represent evidence for a climacteric rise in ethylene production by intact leaves.





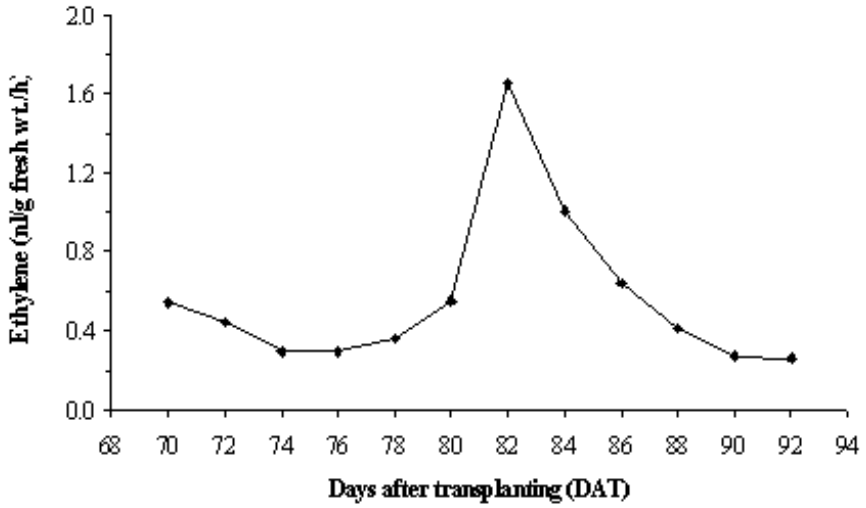


Figure 1. Changes associated with age in dry weight, chlorophyll content (as measured by SPAD value) and ethylene production in leaves of tobacco (values are means of four replications).

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