PLANT PHYSIOLOGY

Vol. 41 No. 7

September 1966

Senescence Inhibition and Respiration Induced by Growth Retardants and ^{6}N-Benzyladenine[1]

A. H. Halevy[2], D. R. Dilley, and S. H. Wittwer

Department of Horticulture, Michigan State University, East Lansing, Michigan

Received November 9, 1965.

Summary. Senescence of Grand Rapids leaf lettuce was greatly reduced at 3 storage temperatures by post-harvest treatment with N,N-dimethylaminosuccinamic acid (Alar) and 2-chloroethyltrimethylammonium chloride (CCC), but not with ^{6}N-benzyladenine (BA). Conversely, Alar and CCC were inactive on broccoli while BA was markedly effective. The deterioration and discoloration of mushrooms was inhibited by Alar with no effect observed from BA or CCC.

The inhibition of senescence by BA and the growth retardants was not always associated with a reduction in respiration (O_2 uptake, CO_2 evolution). BA stimulated respiration and hastened senescence in leaf lettuce accompanied by a diametrically opposite effect from Alar and CCC. Thus, BA cannot be considered a universal senescence inhibitor. The variability of senescence responses induced by different chemicals on a variety of plant tissues suggests a dissimilar mode of action or a complex interaction with native growth substances.

^{6}N-Benzyladenine (BA) is effective in delaying protein and chlorophyll degradation in detached leaves of various species (11) and in prolonging the storage life of some green vegetables (4,5). It has been suggested that these phenomena are a partial consequence of reduction of respiration (4, 5) via inhibition of respiratory kinases (14). We observed that growth retarding compounds N,N-dimethylaminosuccinamic acid (Alar) and 2-chloroethyltrimethylammonium chloride (CCC) are also active in preservation of chlorophyll in leaves of bean and some other plants, and increase the longevity of some perishable vegetables, cut flowers and mushrooms (8). Relationships between senescence inhibition and respiration behavior of various plant species treated with these diverse chemicals are the subject of this report.

Material and Methods

Greenhouse grown lettuce (*Lactuca sativa*, L. cv. Grand Rapids) and field grown broccoli (*Brassica oleraceae* var. Italica cv. Spartan Early), were utilized and treated within 2 to 4 hours after harvest. Heads were selected for uniformity and dipped momentarily or for 10 minutes into the chemical solutions. Freshly harvested mushrooms (*Agaricus campestris*) were shipped by air from Kennett Square, Pennsylvania and treated 5 to 6 hours after harvest. They were soaked for 10 minutes in the experimental solutions. All solutions including the water control contained 0.01 % Tween-20 (Atlas Chemical Industry, Wilmington, Delaware).

After treatment, plant materials were held at room temperature until the surface moisture evaporated, transferred to 8-liter vessels, and placed in respirometer chambers held at constant temperatures. Each replicate contained 2 to 3 heads of lettuce (with a combined weight of 250 to 350 g), 4 to 5 heads of broccoli (ca. 150 g) and 10 mush-

[1] Journal Article No. 3743 from the Michigan Agricultural Experiment Station.

[2] On leave from the Department of Horticulture, The Hebrew University, Rehovot, Israel.

rooms (100–150 g). Three to 4 replicates were employed at each temperature, and each experiment was repeated.

Respiration rates were measured by O_2 depletion and CO_2 enrichment of air flowing (300 cc/min) over the tissue. The O_2 and CO_2 content of the influent and effluent gasses were monitored 2 or 3 times daily by Beckman Model G and Beckman Model 115 analyzers, respectively. Cumulative respiration data were obtained for the intervals specified by approximate integration.

Quality was appraised subjectively when experiments were terminated by 2 to 3 judges using 6 arbitrary grades ranging from 0 to 5, where 0 was excellent (equivalent to freshly harvested), 1 = good, 2 = fair, 3 = poor, 4 = unacceptable, and 5 = badly deteriorated.

Discoloration of mushrooms was estimated by reflectance at 425 mμ, of the outer surface of a disc cut from the center part of the cap. Concentrations for each chemical optimal for the delay of senescence were ascertained by earlier experiments (8).

Results

The comparative respiratory rates of lettuce at 3 storage temperatures and at various intervals after treatment with BA, Alar and CCC are presented in figure 1. The mean quality grades of the lettuce are summarized in table I.

The data in table I indicate that BA had no effect on longevity at 10° and 15° but significantly enhanced the deterioration of the leaves at 20°. BA treated leaves respired significantly higher than control leaves at all temperatures (fig 1A). There was a constant decrease in CO_2 evolution beginning with the first day after harvest in the control followed by leveling off, while in BA-treated leaves there was a prominent peak, followed by a decrease. The time at which the maximum stimulatory effect of BA on respiration occurred was temperature depen-

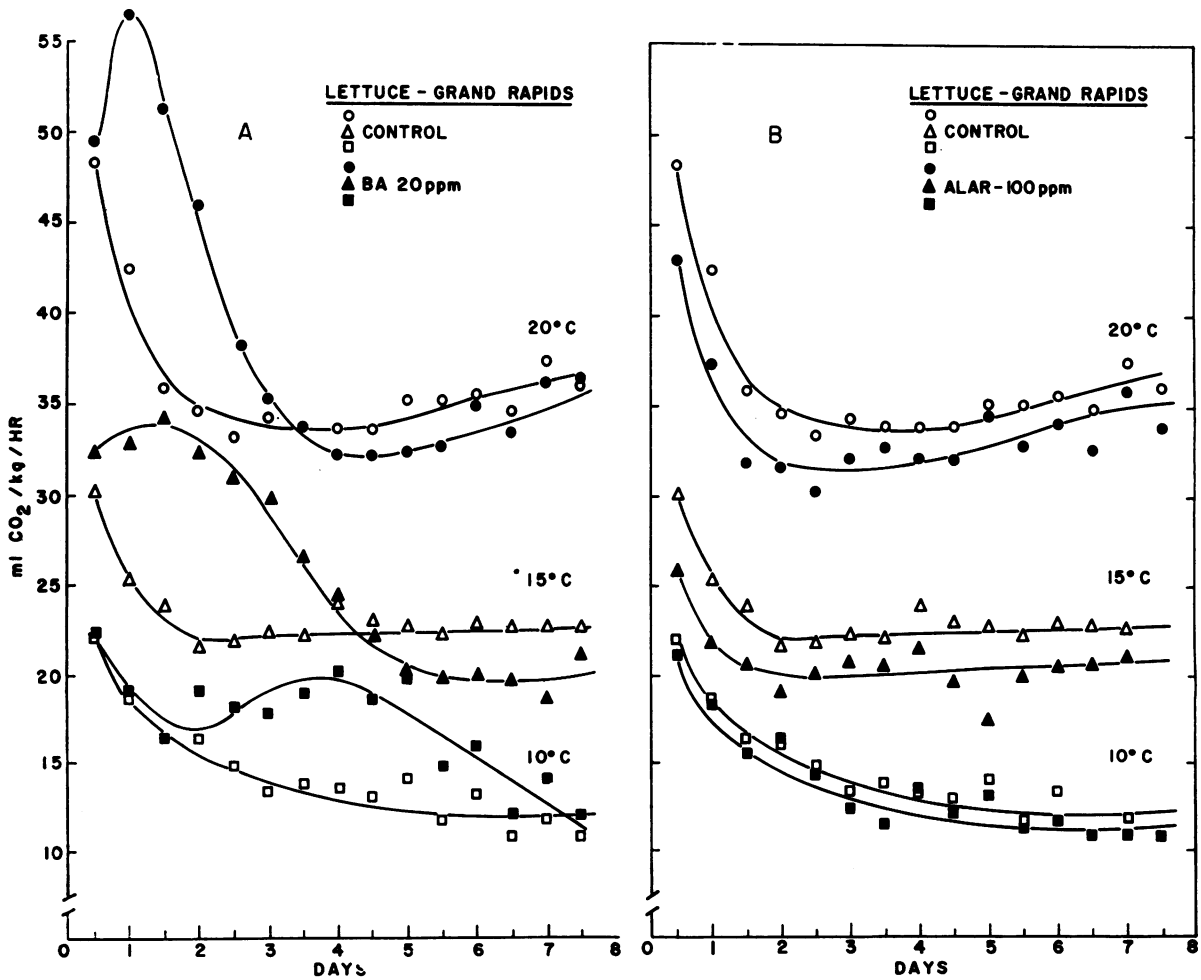

FIG. 1. Respiration rates of Grand Rapids leaf lettuce as influenced by post harvest momentary dip in 8.8×10^{-5} M BA (1A) or 7.2×10^{-4} M Alar (1B) at 3 temperatures. The respiratory response to CCC at 6.2×10^{-4} M was very similar to that of Alar.

Table I. *Influence of BA, Alar and CCC on Quality of Grand Rapids Leaf Lettuce*

Lettuce heads were momentarily dipped in the various chemical solutions, and held at various storage temperatures. Quality grade (O = excellent, 5 = deteriorated) was judged 8 days after treatment.

Treatment	Quality grade		
	10°	15°	20°
Water	3.1 a*	3.9 a	4.1 b
BA 8.8 × 10^{-5} M	2.9 a	3.6 a	4.7 a
Alar 7.2 × 10^{-4} M	0.8 b	2.2 b	2.6 c
CCC 6.2 × 10^{-4} M	1.0 b	2.1 b	2.3 c

* Within each column, values followed by different letters are significantly different (odds 99:1).

dent; being the first day after treatment at 20° and shifting to the fourth day at 10°. Alar and CCC improved the quality grade of lettuce at all temperatures tested (table I). Alar and CCC similarly depressed respiration at 20° and 15° but had no effect at 10° (fig 1B). Since the results with Alar and CCC were very similar, only the Alar data is presented. Neither chemical nor temperature had a significant effect on the respiratory quotients (RQ), which ranged from 0.90 to 0.92.

The results of an earlier report (5) of the effectiveness of BA in delaying senescence and decreasing the respiratory rate of broccoli was confirmed. Alar had no effect on respiration or chlorophyll degradation in broccoli, while CCC increased respiration during the first 48 hours after harvest but did affect chlorophyll degradation.

Alar has been found effective in increasing the longevity and delaying the discoloration of mushrooms over a wide range of concentrations (8). In the present experiments, gibberellic acid (GA), sodium bisulfite (9), BA, Alar and CCC at equal molar concentration, were applied to mushrooms subsequently held at 10° and 20°. There was little or no effect on discoloration with BA, CCC or GA (table II). Bisulfite was slightly effective at 20°

Table II. *Effect of Chemical Treatment on Cumulative Respiration and Discoloration of Mushrooms*

Mushrooms were soaked for 10 minutes in water or in 5 × 10^{-4} M aqueous solution of the chemicals and held at 10° and 20°. Discoloration was measured as percent reflectance at 425 mμ wavelength, 4 days after treatment.

Treatment	CO_2 evolution (liter/kg/30 hr)		% Reflectance at 425 mμ	
	10°	20°	10°	20°
Water	4.39 b*	12.98 bc	29 b	22 c
BA	5.29 a	13.47 a	23 b	22 c
Alar	4.48 b	10.80 c	58 a	43 a
CCC	4.35 b	12.09 d	29 b	24 c
GA	4.95 ab	12.28 bd	30 b	23 c
$NaHSO_3$	4.56 b	11.97 d	31 b	31 b

* Within each column, values followed by different letters are significantly different (odds 99:1).

and ineffective at 10°. Alar, however, markedly delayed senescence and browning at both temperatures. The respiratory rate of Alar treated mushrooms was reduced at 20° but not at 10° (fig 2). BA promoted CO_2 evolution at both temperatures. CCC and $NaHSO_3$ slightly reduced the respiratory rate but only at the higher temperature. No chemical treatment affected the RQ which ranged from 0.87 to 0.89.

Discussion

Inhibition of senescence by BA is often accompanied by a reduction in respiration. It has been suggested that the increased longevity may be a consequence of the decrease in respiration (4, 5) via inhibition of glycolytic kinases (14). The present experiments suggest far greater complexities. While the reduction in respiration and a delay in senescence of BA-treated broccoli (5) was confirmed, an increase in respiration and hastening of senescence was observed for leaf lettuce and mushrooms. BA stimulation of respiration in leaf lettuce occurred early during the holding period and then equalled or dropped below that of the nontreated leaves. This response is opposite to that observed for tissues in which senescence is delayed (4, 5). The results of experiments reported herein with the growth retardants, Alar and CCC, demonstrate that chemical inhibition of senescene is not always accompanied by a concomitant reduction in respiration.

The effect of the growth retardants on respiration appears specific and is probably indirect. Response is dependent on the chemical, plant organ, species and external conditions of temperature. In view of these results, ^{6}N-benzyladenine cannot be considered as a universal senescence inhibitor.

Alar and CCC were also effective in delaying the senescence of some green leaves and cut flowers, and Alar retarded discoloration of mushrooms (8). CCC, while effective for lettuce and bean leaves and some flower cultivars, was not for broccoli, mushrooms and certain other flower cultivars. Alar was highly effective for lettuce and bean leaves, mushrooms, and some flowers, had little effect on broccoli and asparagus, and no effect on other flower cultivars. Variations in effectiveness of the chemicals were also detected in the preservation of cut flowers and mushrooms according to the time of year the crop was grown. Similar results for mushrooms treated with other chemicals have been reported (9). In a few cases where kinins had little or no effect on senescence, auxins (12), gibberellins (3) or a combination of auxin and phytokinins (2, 6) were effective.

Evidence has been presented that the delay in senescence induced by kinins and auxins is related to protein and nucleic acid metabolism. Perhaps an endogenous balance of auxins, kinins and gibberellins is important in regulating nucleic acid and protein metabolism. This labile balance may be al-

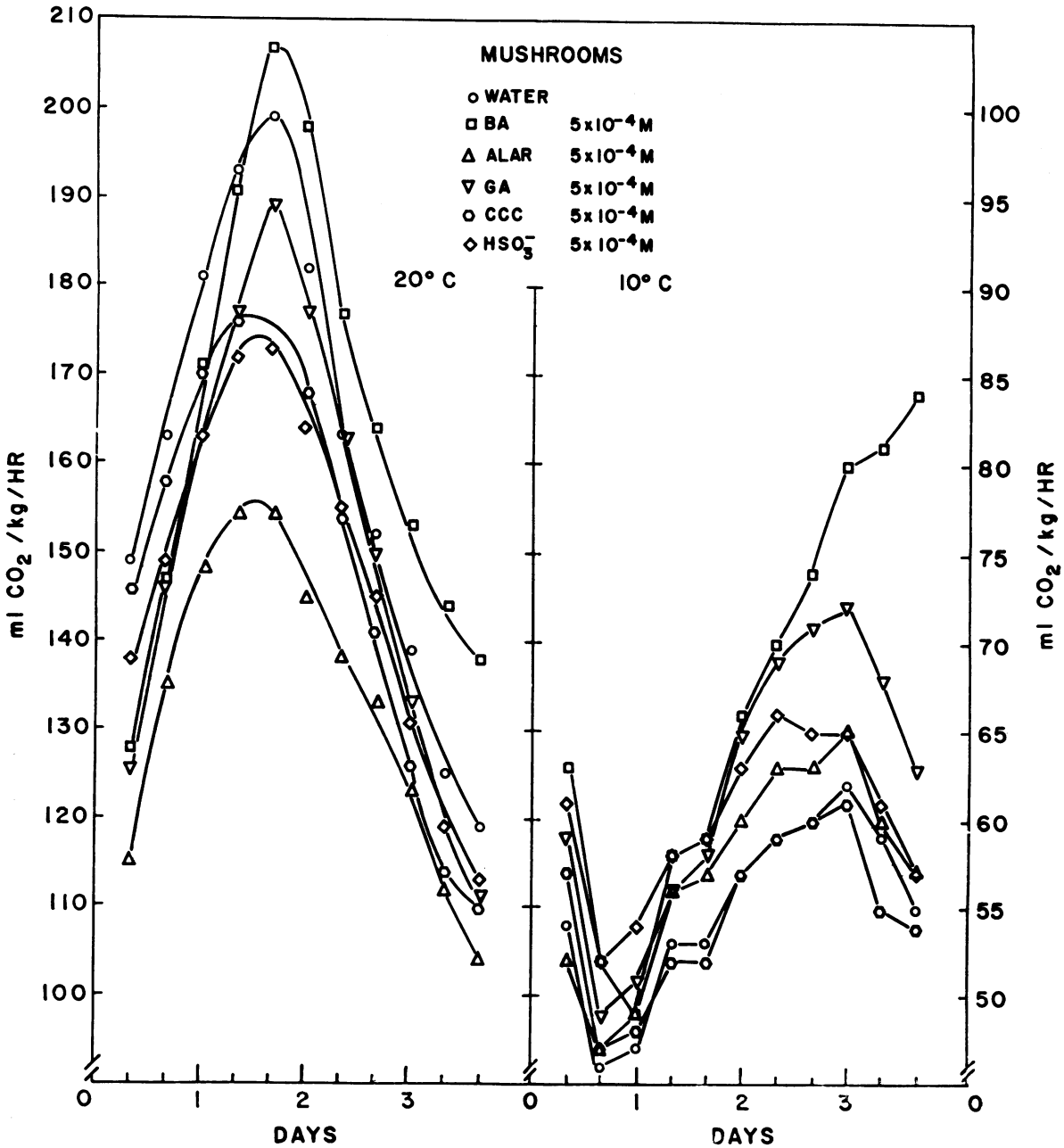

FIG. 2. Respiration rates of mushrooms held at 20° and 10°, as affected by 10 minutes soak in various chemicals at 5×10^{-4} M.

tered after the organ is detached. Endogenous levels of growth regulators differ in various species and cultivars and in organs of the same plant. This balance may vary with organ development and environmental conditions during growth. Such changes may account for selectivity and variability in response of various plants to treatment with chemical stimuli. Growth retardants may reduce the level of both native gibberellins (1) and auxins (10), either by decreasing synthesis (13), increasing destruction (7), or both.

Acknowledgment

The authors gratefully acknowledge the technical assistance provided by Mr. William A. Austin. We also express our appreciation to the American Mushroom Institute, Kennett Square, Pennsylvania, and to Grand Valley Growers, Inc., Grand Rapids, Michigan, for the supply of mushrooms and lettuce, respectively.

Literature Cited

1. BALEDEV, B., A. LANG, AND A. O. AGATEP. 1965. Gibberellin production in pea seeds developing in excised pods: effect of growth retardant Amo-1618. Science 147: 155–57.
2. BALLANTYNE, D. J. 1965. Senescence of daffodil (*Narcissus pseudonarcissus*) cut flowers treated with benzyladenine and auxin. Nature 205: 819.
3. BRIAN, P. W., J. H. T. PETTY, AND P. T. RICHMOND. 1959. Effect of gibberellic acid on development of autumn color and leaf fall of deciduous woody plants. Nature 183: 58–59.
4. DEDOLPH, R. R., S. H. WITTWER, AND V. TULI. 1961. Senescence inhibition and respiration. Science 134: 1075.
5. DEDOLPH, R. R., S. H. WITTWER, V. TULI, AND D. GILBART. 1962. Effect of N^6-benzylaminopurine on respiration and storage behavior of broccoli (*Brassica oleracea* var. Italica). Plant Physiol. 37: 509–12.
6. ENGELBRECHT, L. AND K. CONRAD. 1961. Vegleichende Untersuchungen zur Wirkung von Kinetin and Auxin. Ber. Deut. Botan. Ges. 74: 42–46.
7. HALEVY, A. H. 1963. Interaction of growth-retarding compounds and gibberellin on indoleacetic acid oxidase and peroxidase of cucumber seedlings. Plant Physiol. 38: 731–37.
8. HALEVY, A. H. AND S. H. WITTWER. 1965. Increasing the longevity of perishable vegetables, mushrooms and cut flowers by treatment with growth retardants. Proc. Am. Soc. Hort. Sci. In press.
9. HUGHES, D. H. 1958. Discoloration findings. Mushroom News 4(1): 9-13.
10. KURAISHI, S. AND R. M. MUIR. 1963. Mode of action of growth-retarding chemicals. Plant Physiol. 38: 19–24.
11. MOTHES, K. 1964. The role of kinetin in plant regulation. In: Regulateur Naturels de la Croissance Vegetale. CNRS, Paris. p 131–40.
12. OSBORNE, D. J. AND M. HALLAWAY. 1964. The auxin 2,4-dichlorophenoxyacetic acid, as a regulator of protein synthesis and senescence in detached leaves of *Prunus*. New Phytologist 63: 334–47.
13. REED, D. J., T. C. MOOR, AND J. D. ANDERSON. 1965. Plant growth retardant B-995: a possible mode of action. Science 148: 1469–71.
14. TULI, V., D. R. DILLEY, AND S. H. WITTWER. 1964. ^{6}N-Benzyladenine: inhibitor of respiratory kinases. Science 146: 1477–79.