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## “CURING” OF *NICOTIANA ATTENUATA* LEAVES BY SMALL MAMMALS DOES NOT DECREASE NICOTINE CONTENTS

Ian T. Baldwin<sup>1</sup>

**ABSTRACT.**—Basal leaves of *Nicotiana attenuata* are frequently found neatly excised at the petiole and piled on rocks or soil in the sun until dry, after which they disappear, sometimes to be found again in the nests of *Neotoma lepida*. In response to herbivore attack, *N. attenuata* increases the concentration of nicotine in its leaves, where it functions as an induced defense. Since excision of leaves at the petiole allows for leaf removal without substantially activating this induced defense, and air-drying at high temperatures can volatilize nicotine, we examined the hypothesis that the observed leaf “curing” behavior decreased nicotine contents. In a natural population, replicate bundles of excised leaves were allowed to dry in the sun for up to 96 hours and harvested in 10 intervals. Even though surface temperatures reached 63°C during drying, no significant loss of nicotine was observed. In the laboratory, significant losses of nicotine were not observed until leaves were dried at 100°C. Nicotine contents of naturally “cured” leaf piles at 4 populations were found to be marginally higher than those of neighboring intact plants from which the leaves were likely harvested. We conclude that mammalian “curing” behavior does not reduce nicotine contents and may allow the leaves to be used for insect repellent purposes.

*Key words:* *Nicotiana attenuata*, nicotine, leaf “curing,” hoarding.

*Nicotiana attenuata* Torrey ex. Watson (Sol-anaceae) is a post-fire annual of the Great Basin Desert (Wells 1959, Young and Evans 1978, Koniak 1985). A species that mass-germinates after fires, it synchronizes its germination from long-lived seed banks with germination stimulants in wood smoke (Baldwin et al. 1994) and with the removal of germination inhibitors present in the litter of the dominant vegetation (Preston and Baldwin 1999). If the seed bank is large, the post-fire response can produce dense stands of plants that are exploited by a variety of insect and mammalian herbivores. Several mammals, including black-tailed jack rabbits (*Lepus californicus*), mountain and desert cottontails (*Sylvilagus nuttallii* and *S. audubonii*), and mule deer (*Odocoileus hemionus*), have been observed eating stems and leaves (I.T. Baldwin unpublished results).

However, other mammals exhibit a different pattern of leaf removal, one that we have observed in more than 30 separate populations of *N. attenuata* in Utah over the past decade. In these situations, 1–2 basal leaves are neatly excised at the petiole from as many as 10 plants in a 200-m<sup>2</sup> area and piled together to dry on rocks or dark soil in the sun. In 3 large populations during the 1996 field season (described

in Baldwin 1998), this type of folivory accounted for <1% of the leaf area lost to all herbivores, but in 1 population in 1988 it accounted for 17% of all leaf area lost from 400 plants monitored for the duration of the growing season. We have never witnessed this harvesting, but occurrence of the leaf piles is strongly correlated with the presence of rock and ground squirrels (*Spermophilus townsendii* and *S. variegatus*) and white-tailed antelope squirrels (*Ammospermophilus leucurus*). In 1988, when 2 *S. variegatus* individuals were trapped from a population, harvesting immediately stopped. Leaves are harvested gradually over the growing season and invariably disappear from piles when they are dry. Systematic searches of mammal nests in 2 populations where leaf harvesting was observed found dried *N. attenuata* leaves in 3 nests of the desert woodrat (*Neotoma lepida*). Whether the same species that are responsible for harvesting are also using the leaf material is unknown.

*Nicotiana attenuata* produces the neurotoxin nicotine in high concentrations, and these concentrations increase dramatically when plants are attacked by folivores, but even more so when browsers damage stems (Baldwin and Ohnmeiss 1993). However, if leaves are removed

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by careful excision at the petiole, increases in nicotine concentrations in the remaining tissues are kept to a minimum (Baldwin et al. 1998). Since nicotine is synthesized in the roots and transported to the leaves in the xylem stream (Baldwin 1989), leaf nicotine contents cannot increase after excision. When homogenized leaf material is air-dried at 50°C, significant quantities of nicotine are lost due to volatilization (Baldwin 1988), and since soil temperatures frequently rise above 50°C in *N. attenuata*'s habitats, it is possible that the observed leaf "curing" behavior lowers nicotine concentrations.

Secondary metabolites function for plants as attractants and repellants, but animals are known to use a plant's secondary metabolites for a variety of additional purposes. For example, in the western Rocky Mountains, pikas (*Ochotona princeps*) add *Acomastylis rossii* leaves, which contain high levels of phenolics, to their stored hay piles. These secondary compounds appear to preserve the biomass and nutrient level of the plant tissue and are consumed only when phenolic levels have decreased, midway into the typical storage period (Dearing 1997). Here we examined the hypothesis that *N. attenuata* leaves are harvested to minimize nicotine induction and further dried to lower the nicotine contents. Our results clearly disprove this hypothesis.

## METHODS AND MATERIAL

### Site Description

During the 1996 growing season, *N. attenuata* plants were growing in two 1995 burns that had been started by lightning strikes on 1 July (BLM fire R213; 1163 ha burned) and 8 August (R256; 168 ha burned) in juniper-sagebrush habitats of the eastern slope of Apex Mine Mountain in the Beaver Dam Mountain range, located to the west of Santa Clara, Utah. In addition, plants were growing in a 1994 burn (R332; 809 ha burned) that started on 2 July in southwest Nevada near Motoqua, Utah.

### Sampling Natural "Curing" Piles

Samples of leaves in 11 curing piles were collected during the 1996 growing season from the 4 natural burns. One pile in burn R213 was found approximately 1 km from the other

piles in the same burn, and this group was analyzed separately. When a pile was discovered, adjacently growing plants were inspected and leaf samples were collected from plants from which the leaves in the curing piles could have been harvested. Leaves were collected from stalk positions both above and below the harvested leaf positions.

### Experimental Leaf Curing

Basal leaves (165) from 38 plants were harvested from plants growing in a plantation at Brigham Young University's field station, Lytle Preserve, Beaver Dam Wash, Utah. Leaves were haphazardly assembled into 33 five-leaf piles and laid out on rocks in the sun at the Lytle Preserve. Three haphazardly selected leaf groups were harvested for water and nicotine contents at each of 11 collection times (see x-axis of Fig. 1), including the control harvest at time 0. Soil temperatures were determined with a soil thermometer (Forestry Supplies, Inc.) at various times (see Fig. 1) during the 96-hour experimental period.

To determine the temperature at which nicotine volatilizes from intact, freshly harvested basal leaves (compared with homogenized leaf material; see Baldwin 1988), basal leaves were collected from greenhouse-grown plants, placed in aluminum foil weighing boats, and dried in an electric convection oven at 42°, 50°, 75°, or 100°C. For each drying temperature 3 replicate samples of 3 basal leaves each were dried, and 3 replicate control samples were harvested and freeze-dried to determine maximum nicotine levels (control; Fig 2).

After collection, leaf samples were prepared for nicotine analysis by High Pressure Liquid Chromatography as described in Baldwin (1998). Data were analyzed by ANOVAs, or paired *t* tests.

## RESULTS

More than 80% of a leaf's water content was lost by the 24-hour harvest of the experimental curing piles (Fig. 1, inset), and water contents remained unchanged for the duration of the experiment after the 24-hour harvest. Nicotine contents did not change significantly ( $P = 0.86$ ; Fig. 1) over the 11 harvests of the experiment, and contents were not correlated with soil temperatures.

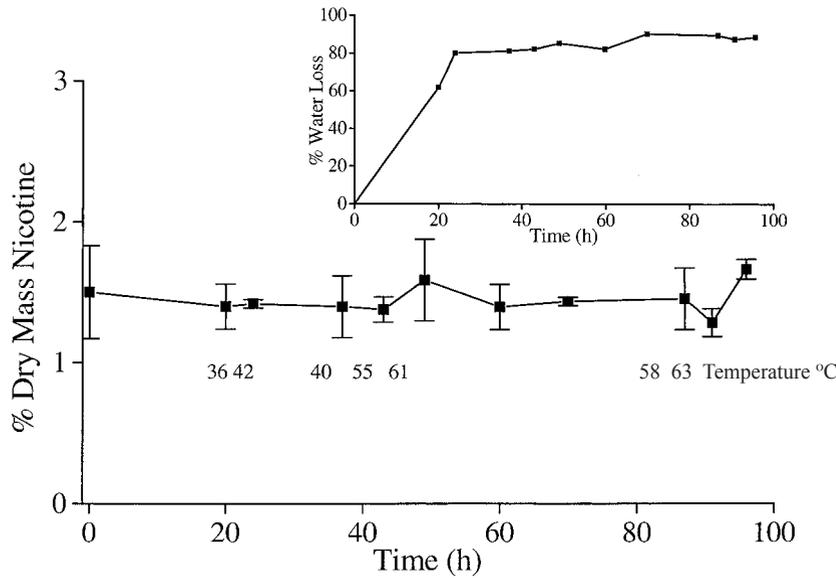


Fig. 1. Mean percentage ( $\pm s$ ) nicotine content for leaves experimentally “cured” on rocks in the sun at the Lytle Preserve for different lengths of time. Thirty-three piles of 5 *Nicotiana attenuata* leaves each were placed on the rocks and harvested at different times, with 3 piles, haphazardly selected, at each harvest. Numbers below selected data points indicate surface temperatures ( $^{\circ}\text{C}$ ) on which the leaves were placed. **Inset** shows the mean percentage water loss of the 3 harvested *N. attenuata* leaf bundles at each collection time.

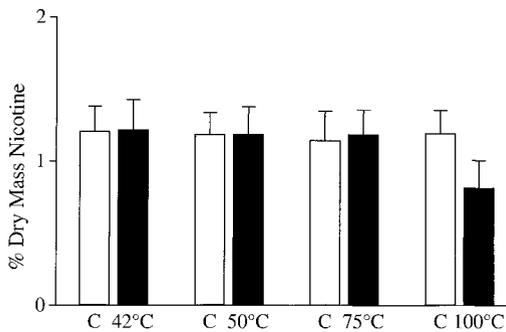


Fig. 2. Mean percentage ( $\pm s$ ) nicotine content for leaves before (open bars) and after drying in a convection oven at 42 $^{\circ}$ , 50 $^{\circ}$ , 75 $^{\circ}$ , or 100 $^{\circ}\text{C}$  (solid bars). Leaves were collected from greenhouse-grown plants and placed on aluminum foil weighing boats with 3 replicates for each temperature. Control samples were freeze-dried before nicotine analysis by HPLC.

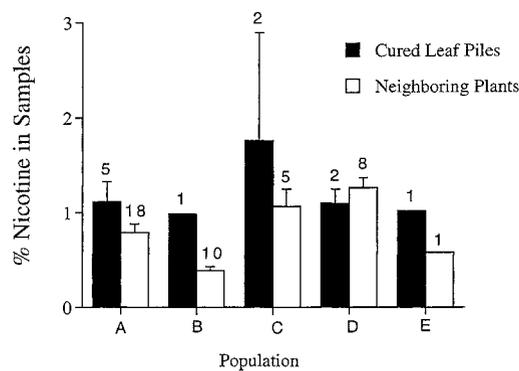


Fig. 3. Mean percentage ( $\pm s$ ) nicotine content of leaves found in “curing” piles (black bars) or in leaves collected from neighboring plants from which leaves had been harvested (open bars) in 4 populations in Utah and Nevada. Samples B and C are from the same large burn (fire R213) but separated by almost 1 km. Numbers above each bar indicate the number of replicate samples from each population.

Intact leaves did not lose significant quantities of nicotine (all  $P > 0.7$ ) when dried at temperatures of up to 75 $^{\circ}\text{C}$ , but when dried at 100 $^{\circ}\text{C}$ , leaves lost 31% of their nicotine contents ( $P < 0.001$ ; Fig. 2).

Leaf samples collected from natural “cur-

ing” piles were marginally higher when “pile” samples were compared against the mean of the values from the basal leaves of neighboring plants ( $F_{1,10} = 4.22$ ,  $P = 0.07$ ; Fig 3). No significant differences were found amongst populations.

## DISCUSSION

Results clearly falsified the hypothesis that the "curing" of *N. attenuata* leaves reduces nicotine contents. Temperatures required to volatilize significant quantities of nicotine from intact leaves (100°C; this study) are clearly greater than those for homogenized leaf material (50°C; Baldwin 1988). Nicotine is thought to be sequestered in the central vacuole, predominantly in epidermal cells (Saunders and Bush 1979), and homogenization by grinding leaves in liquid N<sub>2</sub> may rapidly externalize the nicotine where it is more easily volatilized. Apparently, soil temperatures that were in excess of 60°C during the study were not sufficient to lower nicotine contents.

The observation that nicotine contents of leaves in "curing" piles tended to be higher than the mean of phyllotactically adjacent leaves not harvested from the same plants suggests that the mammalian harvesters were selecting leaves with high nicotine content. However, nicotine contents cannot be the only determinant of leaf choice since nicotine content increases with stalk position on *N. attenuata* plants (Baldwin and Ohnmeiss 1993), and selection of smaller leaves growing at high stalk positions, leaves not normally found in curing piles, would maximize nicotine concentrations. Regardless, leaves are being cured in a way that preserves nicotine contents, and hypotheses should be considered for the function of this behavior.

Many bird species are known to add fresh, aromatic leaves to their nest material (reviewed in Gwinner et al. 2000), an action that may function to reduce ectoparasite loads on nestlings (Wimberger 1984, Clark and Mason 1985) or otherwise enhance the condition of nestlings through unknown pharmaceutical means (Gwinner et al. 2000). The strong insecticidal activity of nicotine (Schmeiz 1971) suggests that *Neotoma* and rock, ground, and antelope squirrels may derive similar benefits from incorporating cured *N. attenuata* leaves into their nests, but this hypothesis requires further testing.

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