Chrysomela scripta, *Plagiodera versicolora* (Coleoptera: Chrysomelidae), and *Trichoplusia ni* (Lepidoptera: Noctuidae) Track Specific Leaf Developmental Stages

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ABSTRACT It is well recognized that host-specialized folivores prefer to feed on young in comparison to old leaves. However, the capacity of young leaf feeders to track specific leaf developmental stages has not been clearly demonstrated. Using three insect folivores and two plant species, we show that nitrogen (N) fertilization changes leaf development and that herbivores track these changes in leaf development. *Nicotiana tabacum* L. and *Populus deltoides* Bartram were fertilized at two and three rates of N addition, respectively. Plants with high rates of N supply had faster growth, greater leaf area, and faster leaf initiation rates than plants receiving low rates of N supply. Most important, all N addition treatments changed the position on the stem where leaves reached 95% full expansion; with leaves on plants receiving high rates of N addition reaching 95% full expansion further from the stem apex (e.g., leaf position 5 versus leaf position 3). Feeding assays with *Trichoplusia ni* Hubner on *N. tabacum*, and *Plagiodera versicolora* Laicharting and *Chrysomela scripta* F. on *P. deltoides* showed that these insect species preferred to feed on leaves at a specific degree of leaf expansion. This preference was exhibited independent of leaf position and N addition rate.

KEY WORDS *Populus deltoides*, *Nicotiana tabacum*, leaf age, leaf position, herbivore feeding, nitrogen fertilization

SUBSTANTIAL EVIDENCE SHOWS that many insect herbivores prefer "young," more apical leaves over "mature," more basal leaves (Cates 1980; Coley 1980, 1983; Crawley 1983; Raupp and Denno 1983; Lowman 1985; Aide 1993; Coley and Barone 1996). However, leaf preference based upon chronological or positional criteria do not reflect the fact that leaf development can change nonlinearly with respect to plant and leaf chronology, plant size, or shoot position in almost all plants with apical meristems (Turgeon 1989, Poethig 1990, Dickson and Isebrands 1991). Consequently, studies using broad, general categories of "young," apical or "old," basal leaves do not reveal if, how, or why insects respond to changes in leaf development (Crawley 1997).

Leaf morphology, physiology and biochemistry change very rapidly in early developmental stages (unfurling and early expansion), begin to slow as leaves approach full expansion, and then stay more or less constant until senescence (Larcher 1980, Myer and Montgomery 1987, Aide 1993). Plants growing under different environmental conditions can have different leaf initiation and expansion rates (Dale 1988, Wait et al. 1998, Wait et al. 1999). Consequently, leaves of the same chronological age or at the same leaf position on a shoot on plants grown under different environmental conditions can be morphologically, physiologically, or biochemically quite different because they are at different developmental stages (Coleman 1986, Dickson and Isebrands 1991, Kursar and Coley 1991) independent of the direct effects of the growth environment on leaf chemistry (e.g., Wait et al. 1998). This is important because leaf morphology, physiology and biochemistry are key characteristics determining folivore food preference (Mattson 1980, Larsson et al. 1986, Myer and Montgomery 1987).

A study by Wait et al. (1998) that examined how nitrogen (N) fertilization of *Populus deltoides* Bartram affected *Chrysomela scripta* F. and *Plagiodera versicolora* Laicharting consumption rates and preference as a function of leaf chemistry illustrates this interaction between leaf development and growth environment. That study showed that changes in leaf chemistry caused by N fertilization were not directly responsible for changes in consumption. Instead, N fertilization altered leaf development, and the changes in leaf development altered feeding behavior independently of the direct effects of fertilization on leaf N and phenolic content. Those experiments used

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leaves of only three leaf positions (leaf plastochron index [LPI] 3, 4, and 5; *sensu* Larson and Isebrands 1971) separating each leaf position postpriori into a developmental stage classified according to stage of expansion (rapid expansion, completing expansion, ceased expansion). The experiments suggested, but did not clearly demonstrate, that beetles could track changes in leaf development.

A more detailed understanding of the relationship between herbivore feeding preference and leaf developmental-stage is important because it could enhance our ability to predict how changes in plant growth in response to the environment affect distributions and amounts of herbivory (Coleman 1986, Crawley 1997, Wait et al. 1998). Here we report experiments designed to specifically test if changes in leaf development caused by N fertilization result in predictable changes in feeding behavior. In particular, we determined whether or not three herbivore species could track a specific window of leaf development, independent of any direct effects of N fertilization on leaf preference. We used the same system and fertilization methodology as in Wait et al. (1998), consisting of *C. scripta* and *P. versicolora* on *P. deltoides*, with exponential N fertilization additions, adding an additional plant and one of its generalist herbivores – *Nicotiana tabacum* and *Trichoplusia ni.*

We knew from a previous study (Coleman 1986) that *P. versicolora* prefers to feed on leaves of 1-yr-old fertilized *P. deltoides* at or around 95% full expansion (LPI 5 in that study). However, it should be noted that LPI 5 is not always going to be the LPI at 95% full expansion (see Wait et al. 1998). Similarly, a study by Bingaman and Hart (1992) showed that *C. scripta* prefers to feed on leaves that are between 40–60% fully expanded (LPI 3 in that study), with this preference occurring across many different *Populus* clones. However, whether that relationship changes with growth environment has not been established in that system. Here we predicted that changes in leaf development induced by N fertilization would result in leaves of the same developmental stage being at different leaf positions on the stem across all treatments; and that the two species of herbivores would track specific leaf developmental stages across treatments irrespective of leaf position. In other words, the herbivores would always prefer leaves of the same developmental stage irrespective of their shoot positions, size, or chronological age. Since we argue that herbivore tracking of leaf developmental stage should be a very general phenomena, we made the same two predictions for *T. ni* on tobacco.

Materials and Methods

Trichoplusia ni* on *Nicotiana tabacum. Seeds of *N. tabacum* variety LAFC 53 were obtained from I. Baldwin at SUNY-Buffalo, Buffalo, NY, and were sown in a 1:1:1 mixture of peat, perlite, and vermiculite. After seedlings had produced two true leaves, 20 plants were transplanted into 1-liter pots in a sand medium (see Wait et al. 1996) and were grown in a greenhouse at Syracuse University, Syracuse, NY. All pots were initially saturated with a modified Hoagland solution (3.5 ml $MgSO_4$, 3.5 ml micronutrients, 3.5 ml PO_4, 0.875 g $CaSO_4$, 0.105 g iron chelate, 0.0035 g KNO_3, 3.5 liter H_2O). Plants were then given 50 ml of this solution daily for 30 d, with N in the solution being added as KNO_3 in an exponentially increasing manner, such that the relative rate of addition would match the relative growth rate of the plant (Ingestad 1982, Wait et al. 1996). There were two N addition rate treatments, with increasing relative rates of addition of KNO_3 of 4 and 12%/d.

Plant height, number of leaves, and the length and width of every leaf on a stem were measured every 2 d on five plants per treatment for 20 d before the herbivore assays following the methods of Wait et al. (1998). Plant height was used to calculate relative height growth rates (cm/cm/d), counting the number of leaves on each plant was used to calculate leaf initiation rates (leaves per day), and leaf area measurements were used to calculate leaf expansion rates (cm^2/d). Height was measured from a paint mark on the base of a plant to the growing tip. A newly initiated leaf was defined as the first leaf from the apex with unfurled edges greater than 2 cm in length (Larson and Isebrands 1971, Coleman 1986) and designated as leaf position 0, the index leaf. Therefore, leaf position 0 is the index leaf from which all other leaf positions are determined. Leaf area was calculated using the following equation: leaf area = 0.0432 + 0.649 * (leaf length * leaf width) ($r^2 = 0.99, n = 142$). The equation was derived from determining leaf area, using a leaf area meter (LI-3100; Li-Cor, Lincoln, NE), of harvested *N. tabacum* leaves grown in a pilot N addition experiment. To test for the effects of nitrate addition on leaf development, the leaf position (relative to index leaf, leaf position 0) where leaf expansion ceased was recorded for leaves produced at the sixth, seventh, and eighth nodes. In addition, leaf expansion curves were constructed for leaves produced at the eighth node to determine and illustrate how leaf area expansion changed as leaves went through their leaf developmental sequence (see Fig. 1A). Third-order polynomials were fit to describe this relationship ($R^2 >$ 0.99 in all cases) and the position where leaves were 95–100% expanded was calculated from the polynomials (Larson and Isebrands 1971).

Trichoplusia ni eggs were obtained from the Geneva Experiment Station, Geneva, NY. Eggs were allowed to hatch into a pinto bean medium provided by the supplier (pinto beans, brewers yeast, ascorbic acid, methyl p. hydroxybenzoate, sorbic acid, aureomycin, propionic acid, Na salt agar, distilled water, vitamin solution, formaldehyde). Experiments began 6 d after the larvae (third instar) emerged from the top of the medium and 20 d after plant growth measurements were initiated. Feeding assay methods and analysis followed the methods of Jones and Coleman (1988). Three third instars were placed into a petri dish arena containing three 2.4-cm^2 disks each of leaves at leaf positions 2–6 (15 discs total). The arenas were lined with filter paper and kept moist. There was one arena

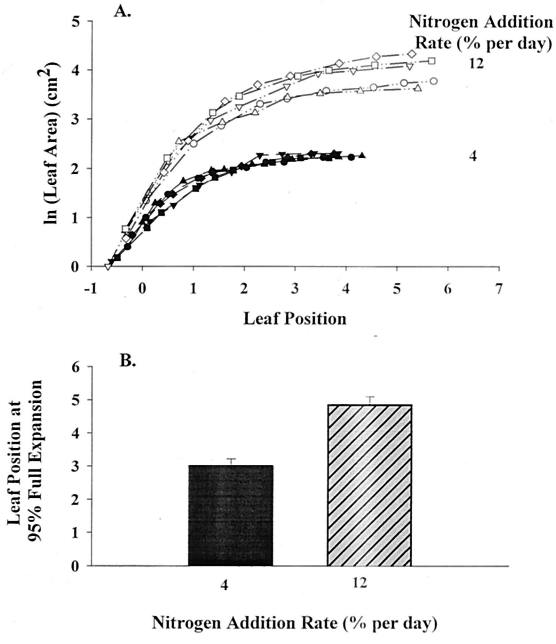

Fig. 1. Effects of nitrogen addition rate on leaf expansion of *Nicotiana tabacum* for leaves produced at the eighth node from the base as a function of leaf position (calculated as a continuous variable following Larson and Isebrands (1971). Open symbols 12% and filled symbols 4% nitrogen addition rate, respectively. Different symbols represent different plants (A). The position on the stem where leaves reached 95% full expansion (a discrete variable, where the index leaf [see *Materials and Methods*] is leaf position 0). Bars are means ± SE ($n = 5$) (B).

per plant and five replicate arenas per N addition treatment. Larvae were allowed to feed for 48 h or until ≈7.2 cm^2 were eaten. The amount fed on each leaf disk was determined with a dot grid every 2 h. Leaf discs did not exhibit significant shrinkage until 30 h after assays began, and most of the feeding was completed (and recorded) before significant shrinkage. Although whole-plant assays (see below) are probably preferable to testing leaf developmental tracking for a number of reasons (e.g., induction) we believe leaf developmental age effects can be determined using disc assays. For example, see Jones and Coleman (1988) where disc and whole plant assays produced the same results. Furthermore, we know that ontogenetic effects on feeding show up relatively clearly (e.g., Wait et al. 1998), and the early literature on ontogenetic effects is based primarily on disc assay results.

***Plagiodera versicolora* and *Chrysomela scripta* on *Populus deltoides*.** Hardwood and softwood cuttings of *P. deltoides* (Clone ST109, obtained from T. Filer (USDA, Southeastern Forest Experiment Station, Stoneville, MI) were rooted in Perlite, transferred to pots (4 liter) in greenhouse soil mix (equal amounts of peat, Perlite and Swiss Farms soil mix (Hyponex, Philmont, NY) and grown at the Institute of Ecosystem Studies (IES), Millbrook, NY, in a greenhouse. Before experiments, plants were fertilized every 14 d (Rapid-Gro [23 N-19 P- 17K] Rapid Gro, Dansville, NY). Nutrient additions were then started using plants that had five leaves. Plants were supplied with 100 ml of an exponentially increasing concentration of N (1:4 ratio NH_4:$N0_3$) every other day for 30 d at a rate of 2, 4, or 6%/d. The initial N solution contained 0.0005 g N. A balanced supply of all other required nutrients was added in proportion to N with mole ratios of N:P:K:Ca:Mg:S of 1.0:0.24:0.06:0.50:0.05:0.04 (see Wait et al. 1996).

Plant height, the length of every leaf, and number of leaves was recorded every 4 d on 10 plants per N addition rate treatment following the methods of Wait et al. (1998, and see above). Leaf area was calculated from the following equation: leaf area = −15.006 + 7.2447 * leaf length ($r^2 = 0.95$, $n = 150$). The equation was derived from harvested leaves grown in previous N addition experiments (Wait et al. 1996, 1998). The above measurements were used to calculate mean relative height growth rate, leaf initiation rate, and the position of a leaf on a stem where it ceased expanding.

Adult *C. scripta* were obtained from E. A. Hart (Iowa State University, Ames, IA) and cultured in a greenhouse on 1-yr-old *P. deltoides* plants. Adult *P. versicolora* were collected the day of an assay from a single wild population on *Salix nigra* at the IES (see Jones and Coleman 1988). To determine whether adult *Chrysomela scripta* and *P. versicolora* beetles could track leaf developmental changes, beetles were placed in aluminum-screened wooden cages (113 by 113 by 76 cm) at the base of single *P. deltoides* plant following the methods of Jones and Coleman (1988). There were four plants per treatment, one plant per cage, and 10 beetles per plant. *C. scripta* beetles were allowed to feed for 25 h and *P. versicolora* beetles were allowed to feed for 90 h because adults of *C. scripta* are much larger than adults of *P. versicolora* and consume more food (Wait et al. 1998). The amount of feeding on each leaf was then determined using a leaf area

Table 1. Effects of nitrogen addition rate on growth and leaf development of *Nicotiana tabacum* (means ± SE; $n = 5$)

Nitrogen addition rate (%/d)	Relative height growth rate (cm/cm/d)	Leaf initiation rate (leaves/d)	Leaf area of leaves at 95% full expansion (cm^2)
4	0.06 (0.01)a	0.245 (0.15)a	21.06 (3.02)a
12	0.132 (0.02)b	0.390 (0.18)b	141.1 (6.80)b

Numbers in a column with different letters differ significantly (*t*-test $P < 0.05$).

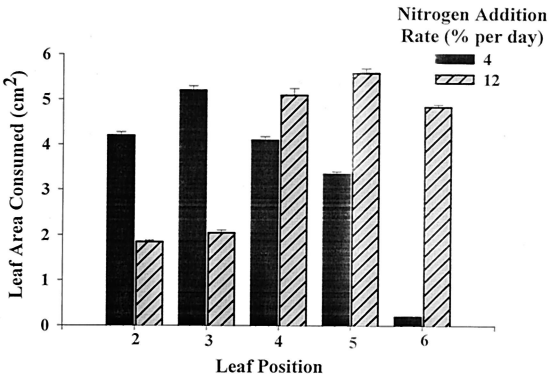

Fig. 2. Consumption of *Nicotiana tabacum* by *Trichoplusia ni* as a function of nitrogen addition rate and leaf position (expressed as in Fig. 1B). Bars are means ± SE ($n = 5$).

meter by measuring the area of a leaf, reconstructing any damaged areas with black tape, and then remeasuring the leaf.

Data Analysis. We analyzed growth and feeding data following the methods of Jones and Coleman (1988), Coleman and Jones (1988a) and Wait et al. (1998). All data were normally distributed. Mean values for the growth rate, leaf initiation rate and leaf position at 95% full expansion of each plant over the growth period before herbivore assays were analyzed using one-way analysis of variance (ANOVA) or *t*-tests. A two-way repeated measures ANOVA was used to examine the effects of N addition rate treatment and leaf position on total consumption. Individual plants were included in the model as a random effect nested within N addition treatment and crossed with N addition rate treatment and leaf position. Statistically significant differences in N treatment or leaf position effects and the interaction between N treatment and leaf position are reported in the text with the resultant *F* value with degrees of freedom for MS and MSE, and *P* value. All statistical analyses were performed using Minitab (12.23, Minitab, State College, PA).

Results

Leaf Development and Growth of *Nicotiana tabacum*. The relative height growth rate, leaf initiation rate, and leaf area at 95% full expansion were all significantly greater in the 12% compared with the 4% N addition rate treatment (Table 1). Leaf expansion curves for leaves produced at the eighth node from the base of plants showed that leaves on *N. tabacum* reached full expansion at different leaf positions (Fig. 1A). An ANOVA showed a significant effect of N addition rate treatment on the leaf position at which leaves reached 95% full expansion ($F = 47.28$; df = 1, 9; $P = 0.0001$; Fig. 1B). Leaves approached the final stages of full expansion when the leaf reached the third node below the apex for all plants in the 4% N treatment, and at the fifth node below the apex for all plants in the 12% N treatment (Fig. 1).

***Trichoplusia ni* Preference for *Nicotiana tabacum*.** Leaf position significantly affected *T. ni* consumption ($F = 4.45$; df = 4, 28; $P = 0.004$), and there was a significant interaction between N treatment and leaf position ($F = 13.64$; df = 4, 28; $P = 0.001$). For plants receiving the 4% N addition rate, *T. ni* fed the most on leaves at leaf position 3 (Fig. 2) – the position where leaves reached full expansion. For plants receiving the 12% N addition rate *T. ni* fed the most on leaves at leaf position 5 (Fig. 2) – the position where leaves reached full expansion. Across all leaf positions assayed, *T. ni* preferred feeding on the three leaves closest to 95% full expansion, with the greatest feeding occurring on the leaf position at 95% full expansion, independent of N addition treatment (Figs. 1 and 2), or growth and developmental rates (Table 1).

Leaf Development and Growth of *Populus deltoides*. Relative height growth rate, leaf initiation rate, and leaf area at full expansion were all significantly greater in the 6% N treatment than in the 4% and 2% N treatments, and significantly greater in the 4% than in the 2% N treatment (Table 2). An ANOVA showed a significant effect of N addition rate treatment on the leaf position at which leaves reached 95% full expansion ($F = 14.25$; df = 2, 29; $P = 0.001$; Fig. 3). A majority of *P. deltoides* leaves in the 2% treatment reached full expansion at leaf position 3; a majority of leaves reached full expansion at leaf position 4 in the 4% treatment, and in the 6% treatment, a majority of leaves reached full expansion at leaf position 5 (Fig. 3).

***Chrysomela scripta* and *Plagiodera versicolora* Preference for *Populus deltoides*.** There was a significant effect of leaf position on *C. scripta* consumption ($F = 10.67$; df = 8, 72; $P = 0.0001$), and a significant interaction between N treatment and leaf position ($F = 3.02$; df = 16, 72; $P = 0.001$). *C. scripta* beetles consumed more leaf area on leaves approaching full expansion than on other leaves (Fig. 4). The majority of feeding occurred on leaf position 1 in the 2% N treat-

Table 2. Effects of nitrogen addition rate on growth and leaf development rates of *Populus deltoides* (means ± SE; $n = 10$).

Nitrogen addition rate %/d	Relative height growth rate (cm/cm/d)	Leaf initiation rate (leaves/d)	Leaf area of leaves at 95% full expansion (cm^2)
2	0.028 (0.002)a	0.216 (0.009)a	12.09 (4.50)a
4	0.035 (0.002)b	0.268 (0.014)b	25.07 (0.80)b
6	0.041 (0.003)c	0.360 (0.015)c	49.9 (1.34)c

Numbers in a column with different letters differ significantly (Tukeys $P < 0.05$).

ment, and full expansion occurred at leaf position 3. The majority of feeding occurred on leaf position 2 in the 4% N treatment, and full expansion occurred at leaf position 4; however, feeding was almost evenly distributed across leaf positions 1, 2, and 3. The majority of feeding occurred on leaf position 2 in the 6% N treatment, and full expansion occurred at leaf position 5; however, a much broader spectrum of leaves were fed upon than in the 2% or 4% N treatments, and there was minimal consumption of leaf position 1 relative to leaf position 2.

There was a significant effect of N addition treatment ($F = 12.34$; df = 2, 72; $P = 0.0001$) and leaf position ($F = 2.65$; df = 8, 72; $P = 0.01$) on *P. versicolora* consumption, and a significant interaction between N treatment and leaf position ($F = 2.74$; df = 16, 72; $P = 0.05$). *P. versicolora* beetles consumed more leaf area on leaves at or approaching full expansion than on other leaves (Fig. 4). The greatest consumption in the 2% N treatment occurred on leaf position 2, the leaf approaching full expansion. The greatest consumption in the 4% N treatment occurred on leaf position 4, the leaf at full expansion. The greatest consumption in the 6% N treatment occurred on leaf position 5, the leaf at full expansion.

Discussion

Evidence for tracking of leaf development. Changes in N availability to *N. tabacum* changed leaf expansion and production rates such that the position on the stem where full leaf expansion occurred was altered (Fig. 1; Table 1). *Trichoplusia ni* clearly tracked those changes because the majority of feeding occurred on leaves that were at or approaching full expansion (Fig. 2), irrespective of leaf position, N addition treatment, or growth and developmental rates. Similarly, changes in N availability to *P. deltoides* changed leaf expansion and production rates such that the position on the stem where full expansion occurred was altered (Fig. 3; Table 2). *P. versicolora* clearly tracked these changes because the majority of feeding occurred on leaves that were at or approaching full expansion (Fig. 4), irrespective of leaf position, N addition treatment, or growth and developmental rates. Although it has been

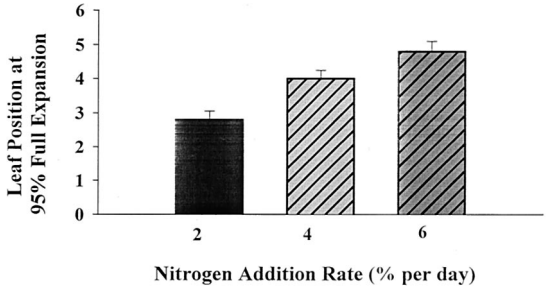

Fig. 3. Effects of nitrogen addition rate on the position on the stem where *Populus deltoides* leaves reached 95% full expansion (leaf position expressed as in Fig. 1B). Bars are means ± SE ($n = 10$).

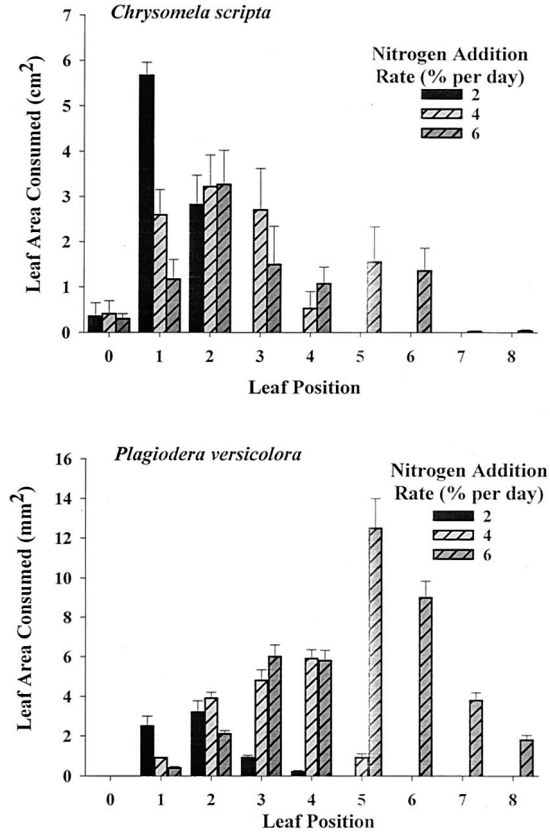

Fig. 4. Consumption of *Populus deltoides* by *Chrysomela scripta* and *Plagiodera versicolora* as a function of nitrogen addition rate and leaf position (expressed as in Fig. 1B). Bars are means ± SE ($n = 4$).

shown that *P. versicolora* prefers leaves at or near full expansion (Coleman 1986, Wait et al. 1998), the finding that this herbivore and *T. ni* track specific leaf developmental stages – measured in terms of degree of leaf expansion – independent of leaf position (or leaf plastochron index), N availability, or growth and developmental rates, is new. *C. scripta* feeding was clearly affected by changes in leaf development, but tracking of a specific leaf developmental stage was only evident between the two lowest N addition rate treatments. *C. scripta* is known to prefer leaves between 40–60% full expansion (Bingaman and Hart 1992, Wait et al. 1998), but evidence of tracking at some N fertilization levels is new.

Tracking of leaf developmental stage was also independent of the effects of N addition treatment on consumption rates. *P. versicolora* consumption was significantly and positively affected by N treatment, whereas *C. scripta* and *T. ni* consumption was not significantly affected by N treatment. Interestingly, leaves at 95% full expansion within a species were at the same chronological age across all N addition treatments because the time it took for a leaf to become fully expanded did not differ between treatments, while leaf initiation rates did (see Tables 1 and 2; Figs.

1 and 3). Therefore, feeding was not independent of chronological age. For example, *P. deltoides* leaves reached full expansion ≈18 d after initiation in all N addition treatments (data not shown). This probably occurred because we fertilized using exponential rates of N addition, which result in relatively constant growth and developmental rates in young plants (Ingestad 1982, Wait et al. 1996, 1998). However, a direct relationship between chronological age and leaf developmental stage does not always occur in N fertilization experiments with these species (D.A.W., unpublished data). Therefore, tracking can be independent of leaf chronological age depending on how leaf initiation rates and developmental rates are related.

How general is the capacity of arthropod herbivores to track such subtle changes in leaf development? Unfortunately, finding an answer to this question from the current literature is difficult because very few studies on plant-consumer interactions have explicitly measured leaf development, and relatively few have measured the preference or performance of consumers on a sufficient number of leaf positions to determine whether or not such a pattern is potentially present (Coleman 1986, Bingaman and Hart 1992, Wait et al. 1998). Nevertheless, there is evidence of leaf developmental stage specialization in a diverse array of insects (e.g., Jepson 1983, Hartnett and Bazzaz 1984, Myer and Montgomery 1987, Coleman and Jones 1988b, Marino and Cornell 1993, Nugent and Wagner 1995, Skinner 1996, Cardoza et al. 2000, Stamp and Bowers 2000), suggesting that the ability of insects to track subtle changes in leaf development might be a very general phenomena.

Significance of Tracking Leaf Development. Our data have important implications for the design and interpretation of experiments with arthropod herbivores that specialize on leaves at specific leaf developmental stages. First, leaf developmental stage and expansion should be determined; for example by either using indexes such as the Leaf Plastochron Index (Larson and Isebrands 1971), or classifying leaves according to their percent full expansion (Wait et al. 1998). Second, when a plant growth treatment affects leaf development, as is known for CO_2 (Wait et al. 1999) or N (Wait et al. 1998) for example, choosing leaves from the same leaf position in arena assays or enclosing herbivores on specific leaf positions can confound interpretation of herbivore behavior. This point can be readily illustrated with data from *T. ni* on *N. tabacum.* If only leaf position 3 were assayed for consumption, one would conclude that the greatest consumption occurred in the low N addition treatment, whereas the opposite conclusion would be drawn if only leaf position 5 were assayed.

Coleman and Leonard (1995) showed the failure to consider leaf developmental stage in plant-herbivore studies can lead to drastic over estimates in the calculation of herbivore consumption. Our results suggest that there may also be far less leaf area that is really suitable for consumption by a herbivore in time and space than would be expected from an assessment based on "young" versus "old" leaves. For example, food quantity estimates for *P. versicolora* feeding on a 15 leaf stage *P. deltoides* shoot are very different if we calculate the leaf area that is suitable or available for consumption based on leaf age classes – as is often done in studies of insect preference as a function of leaf age – versus specific stages of leaf expansion. Based on partitioning leaves into two age classes (young – rapidly expanding, old – fully expanded) and assuming that all young leaves were suitable for consumption and equally preferred, roughly 35% of the total *P. deltoides* leaf area would be suitable for consumption by *P. versicolora* (data not shown, based on data from 2% N addition treatment, Fig. 4). However, we get a very different estimate if the leaf area that is suitable for consumption is based on the specific stages of expansion that are consumed and weighted by preference, where preference can be determined by the relative amount of consumption at a given developmental stage compared with the leaf developmental stage with the greatest consumption (see Fig. 4). In this theoretical scenario, roughly 10% of the total *P. deltoides* leaf area would be suitable for consumption by *P. versicolora* (data not shown, based on data from 2% N addition treatment, Fig. 4). Interestingly, this low value is consistent with the generally low values of consumption by insect herbivores that are observed in nature, where roughly 10% of the leaf material produced in most terrestrial ecosystems is consumed annually by insects (Hodkinson and Hughes 1982).

Along the lines of food quantity, our data also suggest how N fertilization can result in increased herbivore performance independent of changes in leaf quality. For example, the total number of leaves consumed by both *C. scripta* and *P. versicolora* increased with increasing N addition rates (Fig. 4), the leaf area of leaves fed upon increased markedly with increasing N addition rates (Table 2), and the rate at which new leaves went through the developmental stages fed upon increased dramatically as a function of increasing N addition rates (Table 2). These variables expressed together suggest that N fertilization can increase the quantity of preferred (or consumable) leaf area disproportionately. In effect, more than just N fertilization effects due to an increase in total leaf area or N fertilization effects on leaf quality (see Waring and Cobb 1992).

Our data also indicate that the time a leaf is suitable for consumption (i.e., the window of suitability) may be very short and that the time it is suitable is affected by N fertilization. Therefore, it is possible that leaves may only be partially consumed in nature because they have passed through the window of suitability. Partial consumption then could be an artifact of leaf development. Induction studies should carefully consider this artifact, and also examine how leaf developmental stage at the scale examined in this study is related to inducible defenses.

Our study clearly shows that herbivores of different taxa on different host plants track a specific and relatively narrow window of leaf development. The patterns of feeding behavior we observed are likely to be

found across many plant species because they result from an orderly, predictable and highly constrained process of leaf development (Dale 1988); and, many herbivores are leaf developmental stage specialists. If such patterns are found, we may not need to know the specific biochemical attributes of leaves that different consumers use to predict patterns of consumption across insect and plant species (e.g., Wait et al. 1998). Our data also show that changes in N availability to plants alters patterns of leaf production and leaf growth, which in turn, may change the amount of leaf material at a suitable developmental stage for herbivore consumption. These changes may provide a simple, parsimonious explanation of why herbivore performance is generally higher on N fertilized plants (Waring and Cobb 1992) and higher in productive ecosystems (e.g., Vasconcelos 1999), irrespective of any effects of N on the quality of the leaf tissues. In conclusion, the data from this study reinforce the fact that leaf developmental stage has to be carefully considered in the design and interpretation of studies examining herbivore feeding behavior and consumption.

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