Factors Influencing Reproduction of Some Nicotiana Species in the Greenhouse and Field

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A study of physiological responses of *Nicotiana* species to daylength and temperature has been underway here for a number of years. Though most of the species appeared to make satisfactory growth in the greenhouse, about one-fourth have proved difficult to handle (Steinberg 1957, 1959). Satisfactory methods of propagating these had to be worked out while environmental responses were being studied. Moreover, progress in either phase of the work was ... nual replenishment ... her studies. The t... es fell into two groups. The first consisted of large, semi-perennial and woody forms having large leaves. They are native to higher altitudes and tolerant to moisture in well-drained soils. Goodspeed (1954) included them in his Peruvian-Bolivian assemblage. They are referred to here as "Andean." *N. wigandioides* **(Figure 1)** may be accepted as typical of this group. The second group comprises annual and perennial dwarf forms native to arid habitats at higher latitudes. These fall into the Argentine-Chilean assemblage of Goodspeed (1954). They are referred to here as "Patagonian." *N. ameghinoi* **(Figure 2)** might represent this group. The former are short-day plants and the latter long-day ones. The propagation difficulties encountered ranged from seed dormancy or unthrifty growth to failure to flower or form seed, including injury by excessive watering.

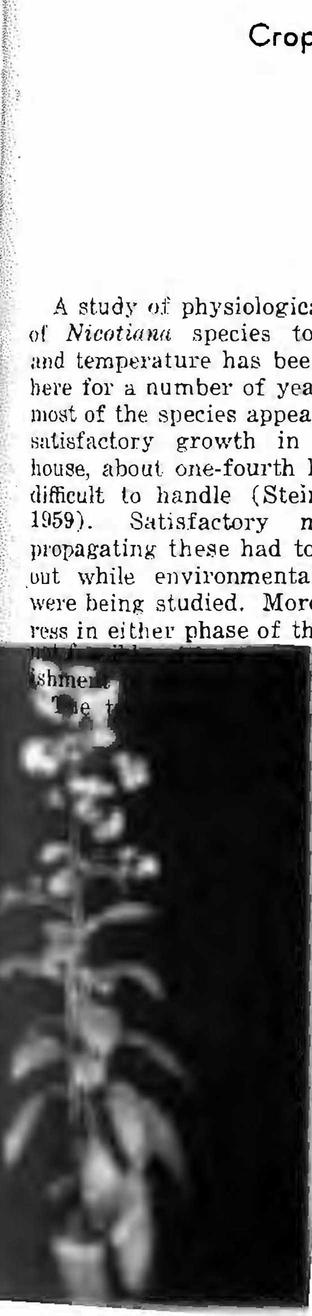

Figure 1. N. wigandioides Koch and Fintelmann, a short-day species from Bolivia.

Emphasis in this report is largely on behavior of species in the Andean group. The phenomena dealt with included the responses of species to season of sowing and to various methods of culture (greenhouse, dark-house, field). Stress is placed on the interrelation between seasonal and other responses in the greenhouse and the environmental requirements of each species for daylength, temperature and moisture.

The revised criteria of photoperiodism of Allard and Garner (1940) are used. The basis for classification of all four categories — short, long, intermediate, and indeterminate (day-neutral)—was altered by them from ability to rapidity of flowering on the daylengths of the 24-hour cycle. The fourth category (indeterminate, or day-neutral) is not used in this article, as such, since short- and long-day plants in *Nicotiana* may give a day-neutral response at suitable temperature levels (Steinberg 1959). "Day-neutral" responses are here considered to represeut minimum sensitivity to day-length, just as "obligate" responses represent maximum sensitivity. A critical daylength for flowering is associated only with obligate responses.

Species desiguations in this report are those accepted as valid hy Goodspeed (1954). Descriptions and authorities, as well as synonymy, of all species are included in his monograph.

Experimental Methods

The experimental procedures employed were similar in most respects to those previously described (Steinberg 1957, 1959). Plants were grown on the short natural daylengths of winter and on a 16-hour daylength

Figure 2. N. ameghinoi Spegazzini, a long-day (?) species from southern Argentina.

Table 1. Effect of month of sowing on season of flowering of some *Nicotiana* species in the greenhouse at 80°F.*

Nicotiana Species	March-sown month of flowering	March-sown days for flowering	June-sown month of flowering	June-sown days for flowering	September-sown month of flowering	September-sown days for flowering	December-sown month of flowering	December-sown days for flowering
Short-day species:								
arentsii	Nov.	239	Nov.	125	Jan.	125	Nov.	311
benavidesii	Mar.	354	Mar.	274	Apr.	236	Oct.	282
glauca	Nov.	163	Dec.	133	Feb.	155	June	164
otophora †	Nov.	219	Nov.	175	Feb.	172	Nov.	314
raimondii †	Feb.	344	Feb.	249	Mar.	182	June	170
setchellii †	Oct.	206	Dec.	195	Mar.	189	Oct.	282
solanifolia	June	102	Nov.	156	Dec.	114	Apr.	128
tabacum	July	110	Oct.	132	Dec.	107	Mar.	111
tomentosa †	Feb.	323	Apr.	356	Feb.	518	Mar.	433
tomentosiformis †	Oct.	213	Nov.	172	Feb.	156	Oct.	293
wigandioides †	Nov.	317	Dec.	207	Dec.	470	Nov.	336
Long-day species:								
alata	June	82	Aug.	76	Mar.	181	Apr.	143
bonariensis	June	84	Oct.	144	Feb.	174	May	177
cordifolia †	Mar.	343	Mar.	260	May	239	June	179
knightiana †	June	84	Sept.	94	Feb.	171	Aug.	235
longiflora	June	69	Aug.	75	Apr.	236	May	172
noctiflora	June	98	Oct.	125	Apr.	183	May	125
petunioides	June	93	Aug.	86	Apr.	209	May	151
repanda	June	75	Aug.	69	Mar.	190	Apr.	142
rustica	June	84	Sept.	84	Nov.	87	Mar.	109
sylvestris	June	89	Sept.	94	May	251	June	183

* *Temperatures of 80°F. often exceeded during June, July, and August. Plants grown in 7-inch pots. The N. tabacum variety was Maryland medium Broadleaf tobacco, Robinson strain, and the N. rustica variety, Brasilia.*
† *Low night temperatures needed for flowering or seed.*

at either a continuous temperature of 75° to 80°F., or a day-temperature of 65° to 75°F. and a night-temperature of 50° to 60°F. These temperature levels could be maintained only when outdoor temperatures were favorable. The 16-hour daylength was produced with incandescent lamps supplementing natural daylengths from 4 AM to 8 PM at an intensity of 30 foot candles (bench). Unless otherwise stated, all plants were grown in seven-inch pots to which was added a gram or so of 4-8-12 fertilizer each week.

The effects of an eight-hour daylength during summer were studied by means of a "dark-house" provided with continuous two-inch wide, slot ventilators at top and bottom. The unit was 5 x 18 and 8 feet tall exclusive of the gable roof. Convection ventilation was ample to prevent any significant rise in temperature even with all doors and roof-slides closed to prevent entrance of light. With the nine side-doors and eight roof-slides open from 8 AM to 4 PM there was little obstruction to natural illumination in the unit. Maryland Mammoth and Stewart Cuban tobacco gave normal short-day responses in this unit.

Additional procedures used to study flowering and seed formation included field plantings and transplanting of vegetative plants to 3½-gallon buckets shortly before frost for further growth in the cold greenhouse. Comparisons were also made of the effects of variation in available soil (seven-inch pots, 3½-gallon buckets) on some of the species. Days for budding and heights at budding and flowering have been omitted to conserve space, though variations were considerable in these respects.

Seasonal Effects in the Greenhouse.—Seasonal effects on flowering of 21 species of *Nicotiana* in the greenhouse are tabulated in **Table 1.** Some of these species are not difficult to propagate, but it was thought desirable to include representative species giving opposite types of daylength response. Of these, only *N. petunioides* **(Figure 3)** might be considered as partaking in some of the characteristics of the so-called "Patagonian group." The data are arranged according to type of daylength response and season of sowing. Except as noted, all species were grown at about 80°F., weather permitting. The excepted species were grown at a day-temperature of 65° to 75°F. and a night temperature of 50° to 60°F. also when weather conditions permitted. These required growth at the lower temperatures in order to obtain comparable data for all four seasonal sowings.

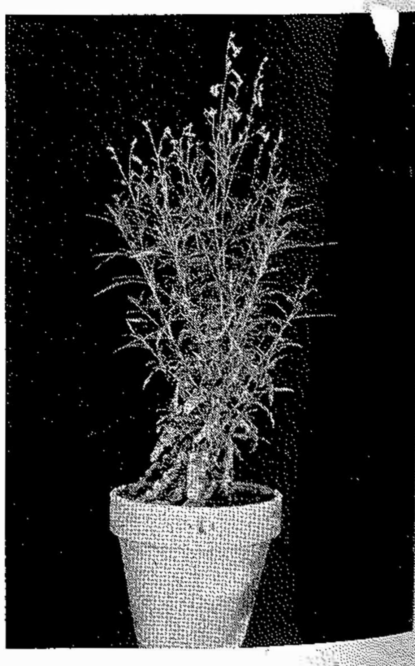

Figure 3. **N. petunioides** (Griesbach) Millan a long-day species from western Argentina and northern Chile.

Table 2. Relation between season and range in rapidity of flowering of sowings in March, June, September and December to type and sensitivity of daylength response.

Short-day *Nicotiana* species	Sowings flowering during Oct-Mar	Flowering* range (±)	Long-day *Nicotiana* species	Sowings flowering during Apr-Sept	Flowering* range (±)
		%			%
arentsii	4	42.7	alata	3	40.8
benavidesii	3	20.0	bonariensis	2	35.6
glauca	3	10.4	cordifolia	2	31.4
otophora	4	29.2	knightiana	3	47.3
raimondii	3	33.9	longiflora	4	54.8
setchellii	4	19.7	noctiflora	3	30.2
solanifolia	2	21.0	petunioides	4	41.7
tabacum †	3	9.7	repanda	3	46.7
tomentosa	3	23.2	rustica**	2	13.0
tomentosiformis	4	30.5	sylvestris	4	47.6
wigandioides	4	36.5			

* *Percentage variation from mean of extremes in time needed for flowering.*
† *Maryland Medium Broadleaf—Robinson*
** *Variety Brasilia*

The number of days needed for flowering of the short-day species with season of sowing (**Table 1**) varied only moderately with three of the species: *N. tabacum*, 25 days; *N. glauca*, 31 days; and *N. solanifolia*, 42 days. With others it varied considerably: *N. arentsii*, 114; *N. raimondii*, 174; *N. tomentosa*, 195; and *N. wigandioides*, 263 days. The long-day species also displayed variation in range of days required for flowering with season of sowing. The ranges in days for flowering varied by only 25, 85, and 93 days for *N. rustica, N. noctiflora*, and *N. bonariensis*, respectively. *N. alata* had a range of 99 days; *N. knightiana*, 151 days; *N. cordifolia*, 164 days; and *N. longiflora*, 167 days.

Blossoming occurred most rapidly in six of the 11 short-day species when sowed in September, in three of the species when sowed in June and in 2 of the species when sowed in March. Only *N. raimondii* flowered most rapidly when sowed in December. Of the 10 long-day species, six flowered most rapidly if sowed in March, three if sowed in June, and one when sowed in December. Flowering of *N. arentsii, N. otophora, N. tomentosa, N. tomentosiformis*, and *N. wigandioides* took place regardless of date of sowing only in the shortest days of the year and that of *N. alata, N. longiflora, N. petunioides*, and *N. sylvestris* only in the longest days of the year.

The data of **Table 1** reveal clearly the type of photoperiodic response and degree of sensitivity to daylength for most species, and agree with previous daylength tests (1959). Type of photoperiodic response is closely correlated with month of initial flowering. Degree of sensitivity to photoperiodic stimulation is indicated by extent of variation in range of days required for flowering. Confusion in classification arises only where sensitivity to daylengths is small (day-neutral responses). Species exhibiting maximum ranges in flowering such as *N. wigandoides* and *N. longiflora* are presumably "obligate" short- and long-day plants, respectively.

These relationships are brought out more clearly in **Table 2** in which the data of **Table 1** are summarized. At least three of the four seasonal plantings with each of the 11 short-day species flowered during October to March, the only exception being *N. solanifolia*. Long-day species analogously flowered in at least three of the four plantings during April to September with three exceptions. The exceptions to characteristic seasons for flowering in the greenhouse are attributed to several causes including lags in flowering after photoperiodic induction and weak sensitivity to daylength stimulus. *N. benavidesii, N. raimondii* and *N. tomentosa*, for example, required as much as 50, 69, and 53 days, respectively, for flowering after flower buds became visible. *N. alata, N. cordifolia*, and *N. knightiana* flower buds have required as much as 73, 79, and 99 days to open. *N. glauca, N. solanifolia*, and *N. tabacum*, on the other hand, are only weakly responsive to daylength. The degree of sensitivity to daylength stimulus is approximately indicated by the percentage variation from the mean between extremes in time needed for flowering of seasonal sowings. Photoperiodic flowering sensitivity of short-day species ranged from probably obligate (*N. arentsii, N. wigandioides*) to almost day-neutral (*N. glauca, N. tabacum*). Long-day species varied similarly from probably obligate (*N. longiflora, N. sylvestris, N. knightiana, N. repanda*) to almost day-neutral (*N. rustica*).

Environment and Flowering.—The effect of environment on time required for flowering of March-sowed species is illustrated in **Table 3.** Duplicate plants of nine different species were grown simultaneously in four different environments. These included the warm and cold greenhouses, an 8-hour daylength (dark-house), and the field. The unflowered plants in the dark-house were brought into the cool greenhouse on October 1 to avoid frost. Two of the ten plants of each species still vegetative in the field were transplanted to 3½-gallon buckets and brought into the cold greenhouse at the same time.

With four exceptions, flowers were formed earlier in the warm than the cold greenhouse. Flowering of *N. cordifolia, N. raimondii*, and *N. wigandioides* was delayed in the warm house, and *N. tomentosa* remained vegetative. However, only *N. cordifolia* and *N. otophora* formed seed in the warm greenhouse, though sparsely. Seed formation in the cold house was usually excellent.

The eight-hour-day regime accelerated flowering and seed formation of *N. wigandioides, N. otophora, N. raimondii, N. tomentosa, N. tomentosiformis*, and *N. arentsii*. Rapidity of blossoming of *N. tomentosa, N. tomentosiformis*, and *N. wigandioides* increased markedly.

Only *N. otophora, N. setchellii*, and *N. tomentosiformis* flowered and formed any seed before frost in the field. *N. arentsii*, however, was in bud. The species still vegetative, when transplanted to 3½-gallon buckets and shifted to the cold section, flowered at about the same time as those kept in the cold house throughout their growth.

These species (**Table 3**) had also been grown to flowering in 3½-gallon buckets in the cold house the previous year and then pruned back after seed formation. The plants flowered again at about the same calendar date as the first flowering. So, also, did the plants that had been sowed a year later.

In other untabulated experiments, duplicate plants sowed in June were grown as usual in seven-inch pots simultaneously with others in 3½-gallon buckets. These differences in

Table 3. Month and days for flowering of some March-sown *Nicotiana* species grown in seven-inch pots in the greenhouse and the 8-hour dark-house, and in the field.*

Nicotiana species	Month and days for flowering of March-sowed plants †											
	In greenhouse continuously				In 8-hr. day (dark-house) until shifted to cold greenhouse†				In field until shifted to cold greenhouse †			
	Warm		Cold		Dark-house		Cold greenhouse		Field		Cold greenhouse	
	Mo.	Days	Mo.	Days	Mo.	Days	Mo.	Days	Mo.	Days	Mo.	Days
arentsii	Oct.	230	Oct.	245	Sept.	197			V	V	Nov.	255
benavidesii	(Sept.)	(354)	Feb.	344	V	V	Oct.	240	V	V	V	V
cordifolia	May	386	Jan.	317	V	V	Feb.	355	**	**	**	**
otophora	Oct.	240	Nov.	257	Sept.	181			Sept.	187		
raimondii	(May)	(411)	Apr.	402	V	V	Jan.	322	V	V	Dec.	296
setchellii	Oct.	220	Oct.	238	V	V	Oct.	233	Oct.	211		
tomentosa	V	V	Mar.	380	V	V	Nov.	252	V	V	Jan.	327
tomentosiformis	Oct.	222	Nov.	248	Aug.	169			Sept.	194		
wigandioides	Jan.	304	Dec.	298	Sept.	184			V	V	Jan.	302

* *Sowed March 5, 1958, except for March 18, 1956, data in parentheses. Non-flowering is indicated as "V."*
† *Treatment with an eight-hour day was begun May 29. Seedlings were transplanted to the field on June 2. Unflowered plants in dark-house were shifted to greenhouse (CS) on Oct. 1, and two each of those in field on Sept. 25, after transplanting to pails.*
** *All ten plants eaten by rabbit or ground hog soon after transplanting to field.*

soil quantity led only to slight and irregular flowering responses on repetition with *N. arentsii*, *N. glauca*, *N. otophora*, *N. setchellii*, and *N. solanifolia*. Use of the larger quantity of soil led to a consistent and marked delay in flowering of *N. cordifolia* (112 days) and a marked acceleration in *N. raimondii* (138 days) and *N. tomentosa* (65 days). *N. benavidesii* and *N. tomentosiformis* also showed slight but consistent increases in rapidity of flowering with an increase in available soil. Flowering of September-sowed *N. knightiana* was hastened by 46 days, but that of *N. glauca* was delayed by 18 days when grown in 3½-gallon pails.

In **Table** 4 are listed the conditions leading to most rapid flowering of the woody semi-perennial species during several years of study. Selection of an inappropriate date for sowing of seed may easily double the period needed for flowering. There is no optimum time for sowing either the short- or the long-day types as a group. Conditions most favorable for rapid flowering of these species are not, however, necessarily suitable for seed formation unless the plants are shifted to a cold environment not later than the early bud-stage.

Watering of plants (soil aeration) has proved difficult to handle in the greenhouse. The standard procedure of frequent light applications of water has sometimes proved unsatisfactory and led to a moist upper soil layer and a bone-dry lower layer in the container. The result was inhibition or death of shallow-rooted species by over-watering and of deep-rooted species by under-watering. Tests with the Andean species revealed that two thorough waterings weekly during winter were almost as effective for growth in size and flowering as one to three light waterings daily.

Table 4. Minimum number of days for flowering of some *Nicotiana* species as affected by season, temperature and daylength.*

Nicotiana species	Date of sowing	Days for flowering	Environment
arentsii	July, Sept	125	WS
benavidesii	Feb.	224	Summer
cordifolia	Dec.	179	CS
glauca	July	133	WS
knightiana	Mar.	84	Summer
otophora	Sept.	169	WS
raimondii	Dec.	170	CS
setchellii	Sept.	180	CS
solanifolia	Mar.	102	Summer
tomentosa	Mar.	252	DH, CS
tomentosiformis	Sept.	144	WS
wigandioides	June	174	WS

* *Abbreviations in this table refer to warm-short (WS), cold-short (CS) and eight-hour dark-house (DH). All plants were grown in seven-inch pots.*

Discussion

The genus *Nicotiana* contains both short- and long-day plants ranging from obligate to indeterminate (day-neutral) in sensitivity to photoperiodic induction of blossoming (Steinberg 1959). Flowering and seeding responses of about half the species also alter variously over a temperature range of about 55° to 75°F. It could be anticipated, therefore, that changes in season of sowing in the greenhouse would greatly influence rapidity of blossoming. A factor in these responses is duration of the juvenile condition in each species. Unless "ripeness to flower"; i.e. maturity, coincides with favorable day-lengths for flowering, the vegetative condition will continue for a maximum of an additional 180 days. This may be the reason for responses indicating effectiveness of preliminary long-days for some of these species as compared to continual short-days in hastening flowering. This situation is, in part, the basis for the wide spread in values for rapidity of flowering in the greenhouse.

Magnitude in range of flowering minima with season in the greenhouse varied roughly with degree of sensitivity to photoperiods.Obligate daylength plants tended to show maximum variations and day-neutral ones minimum annual variations. Flowering of obligate plants in the greenhouse was usually seasonal, whereas that of day-neutral plants was not. The degree of sensitivity to daylength was also evident from the season in which second flowering of old plants occurred. Obligate plants

Tobacco Science, 1959, 3-31, p. 131-135, ISSN.0082-4623.pdf

like *N. tomentosa* remained vegetative until the normal season for blossoming reoccurred. Flowering of these perennial and semi-perennial short-day plants after a previous flowering was generally no earlier than that of seedlings a year younger.

Another condition altering rapidity of flowering with favorable daylengths was soil volume. *N. tomentosa* and *N. raimondii*, when sowed in June, consistently flowered much earlier when grown in 3½-gallon pails than in seven-inch pots, even though fertilizer (4-8-12) was added each week. The gain in rapidity of flowering was 81 days (356-275) for the former and as much as 138 days (361-223) for the latter species. Hastening of flowering by one to two weeks was also regularly observed with *N. benavidesii*, *N. tomentosiformis* and *N. uriganddioides* when sowed in June. *N. cordifolia*, a long-day plant, behaved in an opposite manner to the short-day species, since flowering was retarded in the larger quantity of soil by 112 days (372-260).

It is accepted by many that "root-binding" will hasten flowering. These data indicate that this response is probably not general but depends on variety and season. The utility of these data is decreased by the need for concomitant maturity and inductive daylengths, since maturity could conceivably be hastened by increased soil quantity sufficiently to permit reaction to favorable daylengths before these alter. Growth in four-inch pots (December sowed) hastened flowering only in *N. arentsii* and *N. glauca* (Andean species) under natural daylengths.

The short-day, woody, semi-perennial species of *Nicotiana* flower reliably only in the greenhouse at this location. Moreover, seed formation of field plants is not dependable. Plants should be grown in the greenhouse but can be grown in the eight-hour dark-house or field during the earlier stages of growth. Though high temperature usually increased rapidity of flowering, seed formation required a temperature of less than 70°F., beginning not later than the early bud stage. Manual pollination was always desirable and sometimes mandatory for seed set in the greenhouse. *N. tomentosa*, however, did not flower at 80°F. regardless of daylength, and the others in the Andean group and *N. cordifolia* flowered much less profusely.

Summary

Seasonal responses in the greenhouse with short-day semi-perennial species of *Nicotiana* (Andean group) and typical long-day species when sowed in March, June, September and December were related to type and sensitivity to photoperiod. Short-day behavior of species (Andean group) was checked on an 8-hour day (dark-house). Sensitivity to daylength varied from obligate to day-neutral behavior among short- and long-day species. Greenhouse propagation at temperatures under 70°F. was necessary (at least from the early bud stage) to insure reliable seed formation in species of the Andean group and *N. cordifolia*. Preliminary growth on an eight-hour daylength (dark-house) or in the field before shifting to the greenhouse was satisfactory and even advantageous in hastening flowering in the former case. Increased soil volume was found capable of hastening or retarding flowering depending on species and time of sowing.

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