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Population Variation in Fruit Material of Acer Negundo L.

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POPULATIONAL VARIATION IN FRUIT MATERIAL OF *ACER NEGUNDO* L.

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FRUIT MATERIAL OF ACER NEGUNDO L.

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(Date)

Director of Thesis

Dean of the Graduate College

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INTRODUCTION

Although information is increasing concerning ecological races, Hiesey and Milner (1965) have demonstrated that only a small fraction of the world's plant species have been examined for the presence of populational differentiation. In the species known to have ecological races there is lack of information concerning populations from tropical and temperate or temperate and arctic climates. Responses to photoperiod and thermoperiod of seedlings and seed germination are useful in determining populational differences. Seed germination is of major importance in studying populational differences in that blocks to germination have resulted in natural selection (Toole, et al., 1958). Since populational differences in germination could be responsible for geographical ranges, the comprehension of the ecosystem as well as the establishment and maintenance of populations requires knowledge of seed germination as it relates to the environment.

Acer negundo L., a widespread species, would appear to lend itself to studies of determining ecotypes. Since it has widespread geographical range, several points of investigation are needed to analyze boxelder in relation to mechanisms that such a species would require to live in such an array of habitats. This thesis involves studies of fruit material including fruit weight, seed germination temperature, stratification requirements and the use of bioassays to examine mechanisms of inhibition.

Acer negundo extends throughout the eastern hardwood forest ranging from Canada southward to Central America and westward to California (Harlow and Harrar, 1958; Standley and Steyermah, 1944; Boivin, 1966). It is common to bottom-lands along rivers and streams or on deep moist soil (Tolstead, 1947; Harlow and Harrar, 1958). Boxelder is also found on poorer sites and is perhaps the most aggressive of the maples in maintaining itself in unfavorable conditions (Harlow and Harrar, 1958). It has been shown that is is shade tolerant (Walker, 1957), but is not tolerant to long periods of inundation (Hosner, 1958). Vaartaja (1957) reported that *Acer negundo* showed no significant photoperiodic response among populations.

Acer negundo is not an economically important timber tree. It has been successfully used as a windbreak species (George, 1936), and the seed serves as a food source for both the red squirrel and the evening grosbeak (Stoner, et al., 1939; Brooks, 1956). The eastward extention of the evening grosbeak has been facilitated by the widespread planting of boxelder (Baillie, 1940). Although boxelder is seemingly commercially unimportant, it may become of greater value as man selectively depletes natural resources.

The taxonomy of Acer negundo has been a topic of disagreement in past years. Plowman (1915) brought attention to the morphological and physiological differences between Acer negundo and other Acer species, and, after considering the geological history of Acer negundo, concluded that the taxon should be placed in its own monotypic genus under the bionmial, Negundo aceroides Moench. Hall (1951, 1954) agreed with this placement after his work with the floral anatomy of Acer negundo and other members of the genus Acer. However, it has been pointed out that differences cited by Plowman as occurring in the xylem are too slight to support the change in rank (Metcalfe and Chalk, 1965). Although some workers have used the change to Negundo, the species is generally referred to as Acer negundo L. When the change is used, there appears to be synonomy with N. aceroides, N. negundo (L.) Karst, N. nuttallii (Nieuwl.) Rydb., and N. fraxinifolium Nutt.

There are several varieties of Acer negundo listed, but the ranges of each seem to overlap. Kearney, et al. (1964) reported that the species is represented in Arizona by the variety interius (Britton) Sarg. but that there is intergradation with a variety call arizonicum Sarg. The variety interius is reported nearly throughout the range of boxelder. Apparently there is some confusion as to how many varieties of Acer negundo should be recognized, and revisions may be necessary.

Although all authors indicate that Acer negundo is dioecious, Hall (1951, 1954) has reported the presence of viable stamens in what appear to be pistillate flowers and aborted pistils in what appear to be staminate flowers. Parthenocarpy has also been observed in this species (Beketovskie and Beketovskie, 1935). Since the frequency of both moneclinous flowers and parthenocarpy is apparently low, it can be assumed that populations of boxelder are cross fertilizing.

Lewis (1969) has pointed out that evolution is inseparably related to the ecosystem in which it occurs, but we have remarkably little understanding of the complex interactions within ecosystems and their impact on evolution. The first stage in relating evolutionary process to the ecosystem is the observation of a correlation between differences among populations of similar, apparently closely related organisms and among habitats or areas they occupy. This thesis is a summary of preliminary studies to determine what adaptations have taken place in Acer negundo which enable it to occur over a wide distribution.

METHODS AND MATERIALS

General Procedure

Collections of fruits were obtained with the aid of several individuals from twenty populations ranging from 31.5 degrees (East Texas) to 49.0 degrees latitude (North Dakota). Five populations were located in western Kentucky. Two populations (Canada and Clinton, New York) fell outside the published distribution for Acer negundo (Fig. 1).

Upon arrival, the collections were inspected, cleaned, and dried at room temperature. When dry, the collections were placed in paper containers and stored in the dark at room temperature. A code was assigned to each collection consisting of the state abbreviation, and, in the cases of multiple collections, a number, and a letter. For example, the collection from Bowling Green, Kentucky, was coded Ky-4-A. The number four identifies the location of the population within the state, and the letter identifies the parental tree, "A". Mixed collections from several trees are designated by the letter, "M". Coding, location, and collection site data are given in Table 1.

Three controlled environmental chambers were used in the germination studies. Chamber I was held at 22 $\pm$ 1 C with a maximum light intensity of one-hundred and fifty

Table 1. Collection site data.

Population	Location	Degrees and Minutes Latitude	Degrees and Minutes Longitude	Elevation (feet)	Growing Season(a) (days)
N.D.	Bottineau Co., N.D.	48°51'	100°27'	1,640	127
Can.	Quebec, Quebec	46°75'	71°25'	296	120
Minn.	Washington Co., Minn.	44°51'	93°13'	830	171
N.Y.	Herkimer Co., N.Y.	43°	75°	424	183
Iowa	Floyd Co., Iowa	43°04'	92°40'	1,015	175
Mich.	Livingston Co., Mich.	42°50'	84°	500-1,000	120-160
Ohio	Athens Co., Ohio	39°	82°10'	700	161
Calif.	Solano Co., Calif.	38°	120°80'	500	300-200
Ky.-3	Fayette Co., Ky.	38°	84°50'	500	180-200
Ky.-1	Daviess Co., Ky.	37°75'	87°	500	180-200
Mo.-M	Texas Co., Mo.	37°50'	91°75'	1,300	180-200
Ky.-2	Garrard Co., Ky.	37°39'	84°46'	955	188
Ky.-5	Edmonson Co., Ky.	37°	86°45'	500-1,000	180-200
Ky.-4	Warren Co., Ky.	37°	86°50'	500-1,000	180-200
Tenn.-1	Anderson Co., Tenn.	36°11'	84°04'	500-1,000	180-200
Tenn.-2	Anderson Co., Tenn.	36°11'	84°04'	500-1,000	180-200
Ark.	Washington Co., Ark.	36°	94°	500-1,000	200-300
S.C.	Hart Co., S.C.	34°54'	82°13'	957	225
Geo.	Bibb Co., Geo.	32°75'	83°50'	500-1,000	200-300
Tex.	Angelina Co., Tex.	31°36'	94°35'	308	243

(a) Growing season data from Visher, 1954.

Figure 1. Map of collection sites. (Circles represent the collection sites).


40°
30°
40°
30°

foot candles on a twelve hour photoperiod. Chamber II was held at 11 ± 1 C with a maximum light intensity of one-hundred and fifty foot candles on a twelve hour photoperiod. Chamber III was programmed for a thermoperiod of 30 C day and 18 C night. The light intensity of Chamber III was one-hundred and thirty foot candles on a twelve hour photoperiod.

Bioassays were conducted with Lactuca sativa (cultivar 'Prize Head') and Raphanus sativus (cultivars 'Scarlet Globe' and 'Crimson Giant'). For testing, fifty seeds either of the radish or lettuce were sown on a single layer of filter paper to which the extract or solvent was added. All bioassays were placed in Chamber I (22 C, 12-hr day) and germination counts were made daily.

Physical Data Studies

Fruit Weight. Comparison of fruit weight, in grams per one-hundred seeds, were made among fourteen populations (North Dakota, Canada, Minnesota, New York, Iowa, Kentucky-2-M, Missouri, Kentucky-1, Kentucky-4, Tennessee-2, Arkansas, South Carolina, Georgia and Texas).

Three seed lots, one lot from each parental tree, consisting of one-hundred seeds were removed from each population with the exceptions of Texas, South Carolina and Missouri which were represented by two lots each. The fruits were cleaned, dewinged, and dried at 72 C for twenty-four hours. At the end of the drying period the fruits were weighed on an analytical balance to 0.001 of a gram. Between weighings, the fruits were kept in a desicator over phosporous pentoxide.

Fruit and Wing Length. Comparisons of fruit length (exclusive of the wing), wing length, and total length of the fruit were made among thirteen populations (North Dakota, Canada, Minnesota, New York, Iowa, Kentucky-2, Missouri, Kentucky-1, Tennessee-2, Arkansas, South Carolina, Georgia, and Texas). Fifty seeds were drawn at random from the collections and measured to the nearest millimeter.

Caloric Value. Caloric values were determined for fruits from the same populations used in the fruit weight determinations.

The fruit lots were ground with an Allen Thompson Mill until the particle size would pass through a size forty mesh. The material was made into pellets using a Parr pellet press. Pellets were kept in a desicator to prevent absorption of water, and the pellet weight was determined prior to combustion. Pellets generally were 1.5 x 1.0 mm in size and weights ranged from 0.3 to 1.0 gm.

Analyses for energy content were made by igniting the pelleted fruit material in a calorimeter (Parr Series 1211 Adiabetic). The pellets were ignited with ten centimeters of Parr "Chromed C" fuse wire. Temperatures after firing were allowed to equiliberate for three minutes, and the initial and final temperatures of the water bath were recorded to the nearest 0.01 F.

Corrections for the formation of acids during combustion were made by titrating the washings from the bomb with a 0.0725 normal solution of Na_2CO_3 (Anonymous, 1960). Methyl orange was used as the indicator. Correction for the exothermic heat produced by the fuse wire was determined by measuring the unburned wire and calculating 2.3 cal/cm of burned wire. Caloric value of fruits was recorded as cal/gm.

Six caloric determinations were made per population with the exception of Missouri (three determinations) and Texas and South Carolina (four determinations each).

Germination Studies

Stratification Tests. To determine if *Acer negundo* displayed populational differences in length of stratification period required for germination, the following test was conducted.

Study A: Fourteen populations were used in the first replication (Canada, New York, North Dakota, Minnesota, Iowa, Missouri, Michigan, South Carolina, Kentucky-1, Kentucky-2, Kentucky-3, Tennessee-2, Texas and Georgia) with two-hundred and fifty fruits being removed from each parental tree or mixed collection. The fruits were cleaned, dewinged, and surface sterilized with Semesan. Lots of fifty dewinged fruits were placed in a petri dish on three layers of moistened filter paper. Each dish was placed into one of five groups. The groups of dishes were placed in a light proof container and stored in a 4-5 C chamber for four, six, eight, ten or twelve weeks. At the end of its stratification period each group was moved to a 22 ± 1 C chamber for two weeks. Germination counts were made every three to four days during the two week period.

Study B: In the second replication, twenty populations were used (Canada, New York, North Dakota, Minnesota, Iowa, Missouri, Michigan, Ohio, South Carolina, Arkansas, California, Kentucky-1, Kentucky-2, Kentucky-3, Kentucky-4, Kentucky-5, Tennessee-1, Tennessee-2, Georgia and Texas). The procedure was the same as outlined in Study A above.

Temperature Preference. Material from eleven populations was used to determine if any difference in temperature preference existed among populations. The populations used were Canada, New York, North Dakota, Minnesota, Iowa, Missouri, South Carolina, Kentucky-5, Tennessee-1, and Georgia. One-hundred and fifty fruits were removed from collections, cleaned, dewinged, and separated into three lots of fifty each. Each lot was surface sterilized with Semesan and placed in a petri dish on three layers of moistened filter paper. The dishes were separated into three groups. Each group was placed into a metal light proof container and stratified for twelve weeks at 5 C.

At the termination of the stratification period, the groups were removed from the containers and placed in one of three chambers (Chamber I, 22 C 12 hr day; Chamber II, 11 C 12 hr day; and Chamber III, 30-18 C 12 hr day).

Germination counts were made every three to four days for a duration of two weeks.

Inhibitor Studies

Pericarp. To determine the role played by the pericarp in the primary dormancy of the seed the following studies were conducted.

Study A: Four populations were used in the first study (Iowa, Kentucky-2, Tennessee-2 and Georgia). Two-hundred fruits were removed from each collection and separated into four lots of fifty each. Each lot was then used in one of four treatments.

(1) Removal of the pericarp and placement in Chamber I (22 C 12 hr day).

(2) Retention of the pericarp and placement in Chamber I.

(3) Removal of the pericarp, stratification for four weeks at 4 C and transferral to Chamber I.

(4) Retention of the pericarp, stratification for four weeks at 4 C, and transferral to Chamber I.

In all the treatments the material was surface sterilized with Semesan and placed on three layers of moistened filter paper in petri dishes. Germination counts were made every three to four days for a duration of two weeks.

Study B: Two populations (Kentucky-2-M, and Georgia-M) were used to determine if light was necessary for germination when the pericarp was removed. At the same time, a second test was initiated to ascertain whether an aqueous extract of the pericarp would inhibit seed germination.

One-hundred and fifty fruits were removed from each collection and separated into five lots of thirty each. Each lot was then treated in one of the following ways.

(1) Retention of the pericarp and placement in Chamber I.

(2) Removal of the pericarp and placement in Chamber I.

(3) Removal of the pericarp; placement on filter paper moistened with aqueous pericarp extract; transferral to Chamber I.

(4) Retention of pericarp and placement in the dark in Chamber I.

(5) Removal of the pericarp and placement in the dark in Chamber I.

In all the treatments the material was surface sterilized with Semesan and placed on three layers of filter paper moistened with the proper solution. The aqueous extract of the pericarp was prepared by grinding 1.0 gm of the pericarp material for two minutes in 10.0 ml of distilled water with a Virtis homogenizer. The slurry was filtered through four layers of cheese cloth.

Germination counts were made every three to four days for a duration of two weeks. The material in the dark was counted on the twelfth day only.

Inhibitory Effect of Aqueous Fruit Extract

Study A: An aqueous extract of 3.0 gm of dewinged fruit material was prepared by blending it with 30.0 ml of distilled water in a Virtis homogenizer for five minutes. The slurry was centrifuged to remove the insoluble material. A 2.0 ml aliquot of the extract was used in a lettuce bioassay.

An aqueous extract of the wing material was prepared by blending 1.0 gm with 15.0 ml of distilled water in a Virtis homogenizer for five minutes. Insoluble material was removed by centrifugation. A 2.0 ml aliquot of the supernatant was used in a lettuce bioassay.

Study B: Using Minnesota material, a study was conducted to determine if the inhibitory effect was caused by the pericarp. Fruits were separated into seed and pericarp. Lots of 1.5 gm of each were ground in 15.0 ml of distilled water, surrounded by an ice bath, in a Virtis homogenizer. The slurry was filtered through Whatman (No. 1) filter paper, and 3.0 ml of the aqueous extract of each was used in lettuce bioassays.

Study C: To determine if there was a difference in the amount of inhibitor present on a fresh weight basis a study was conducted using ten populations (Canada-C, Iowa-B, Kentucky-1-B, Kentucky-2-M, Kentucky-4-A, Kentucky-3-D, Missouri-M, Texas-D, and Georgia-M).

Fruit material (3.0 gm) was removed from each collection and ground in 30.0 ml of distilled water, surrounded by an ice bath, in a Virtis homogenizer. The slurry was filtered through Whatman (No. 1) filter paper, and a 3.0 ml aliquot of the extract was used in the lettuce bioassay.

Solubility Test. To characterize the inhibitor a series of solubility tests were conducted using Georgia and Minnesota fruit material.

Study A: A 1.5 gm fruit lot from each population was blended in 15.0 ml of distilled water, surrounded by an ice bath, in a Virtis homogenizer. The slurry was filtered through Whatman (No. 1) filter paper, and the filtrate was washed with an equal volume of ether. A volume of 3.0 ml of both the ether and water extract was used in lettuce bioassays. When a volatile substance, as ether, was used in the extraction, it was allowed to evaporate from the filter paper. The filter paper was then remoistened with an equal volume of distilled water and the lettuce seed was sown.

Study B: The extraction procedure was the same as in Study A with the substitution of chloroform for ether.

Study C: The extraction procedure remained the same as in Study A except the fruit material was ground in 80% methanol (v/v). The methanol filtrate was washed with ether.

Study D: The extract procedure varied in that the material was ground in 80% ethanol (v/v), and the filtrate was washed with petroleum ether.

Inhibitor-Stratification Test. Five populations (New York, Iowa, Kentucky-1, Tennessee-2, and Georgia) were used to determine if the inhibitor effect decreased with length of stratification.

Study A: A sample of 27.0 gm of fruits was removed from each collection and separated into nine 3.0 gm groups. Each group was cleaned, dewinged, surface sterilized with Semesan, and placed on three layers of moistened filter paper in petri dishes. Eight groups were stratified at 4 C with one group being removed each week over the eight week test period; one group was retained as a control.

At the termination of the stratification time, the 3.0 gm of material was washed with distilled water. The material was then ground with 30.0 ml of cold water (5 C) in a Virtis homogenizer. The slurry was divided equally into four 15.0 ml centrifuge tubes. Insoluble material was removed by centrifugation. A volume of 2.0 ml of the supernatant was used in a lettuce bioassay.

Study B: The filter paper that the fruit material was stratified on in Study A was dried overnight at 53 C. The paper was then cut into small strips and eluted with 20.0 ml of distilled water on a rotary shaker. A 3.0 ml aliquot of the elutant was assayed against lettuce seed.

RESULTS

Physical Data Studies

Fruit Weight. Populational differences were evident in fruit weight of fourteen lots collected over a wide range of latitude (Table 2; Figure 2). The fruit weights showed a general trend of increase with an increase in latitude of origin. Only two populations (North Dakota and Missouri) failed to fall in the pattern of heavier fruits from the more northern habitats. Statistical analysis of the fruit weights showed them to be significantly different at the 0.01 level. Separating and pooling the populations above and below 37.0 degrees latitude demonstrated that the fruit material from the northern habitats average 3.57 gm and the material average 2.36 gm. Statistical analysis of the pooled fruit weights showed them to be significantly different at the 0.05 level.

Fruit and Wing Length. From Texas to Kentucky-2-M the southern populations displayed a cline with an increase in fruit length (exclusive of the wing) with increase in latitude (Table 3; Figure 3). The remainder of the populations tested varied greatly from the general trend. Only three populations (Texas, Iowa, and Canada) failed to display intrapopulational variation (Figure 3). The Canada and North Dakota populations did not differ significantly

Table 2. Comparison of fruit weights and caloric value among fourteen populations of *Acer negundo*.

Population	Fruit Weight(a)*	Caloric Value(b)*
N.D.	2.948 ± .167	5,039 ± 124
Can.	4.051 ± .439	4,967 ± 78
Minn.	4.537 ± .663	5,079 ± 126
N.Y.	4.658 ± .679	5,064 ± 105
Iowa	4.047 ± .241	5,084 ± 48
Ky-1	3.046 ± .126	5,352 ± 142
Mo.	1.947 ± .032	4,975 ± 105
Ky-2	3.504 ± .261	5,156 ± 274
Ky-4	3.435 ± .722	4,959 ± 133
Tenn-2	2.962 ± .190	5,163 ± 236
Ark.	2.407 ± .547	5,159 ± 143
S.C.	1.134 ± .109	4,972 ± 163
Geo.	2.879 ± .09	5,144 ± 92
Tex.	1.852 ± .646	5,026 ± 333

(a) Grams per hundred fruits.
(b) Calories per gram.
* ± one standard deviation.

Figure 2. Comparison of fruit weights (gm/100 seed) among fourteen populations of Acer negundo. Mean (circle) and extremes in weight (bar).

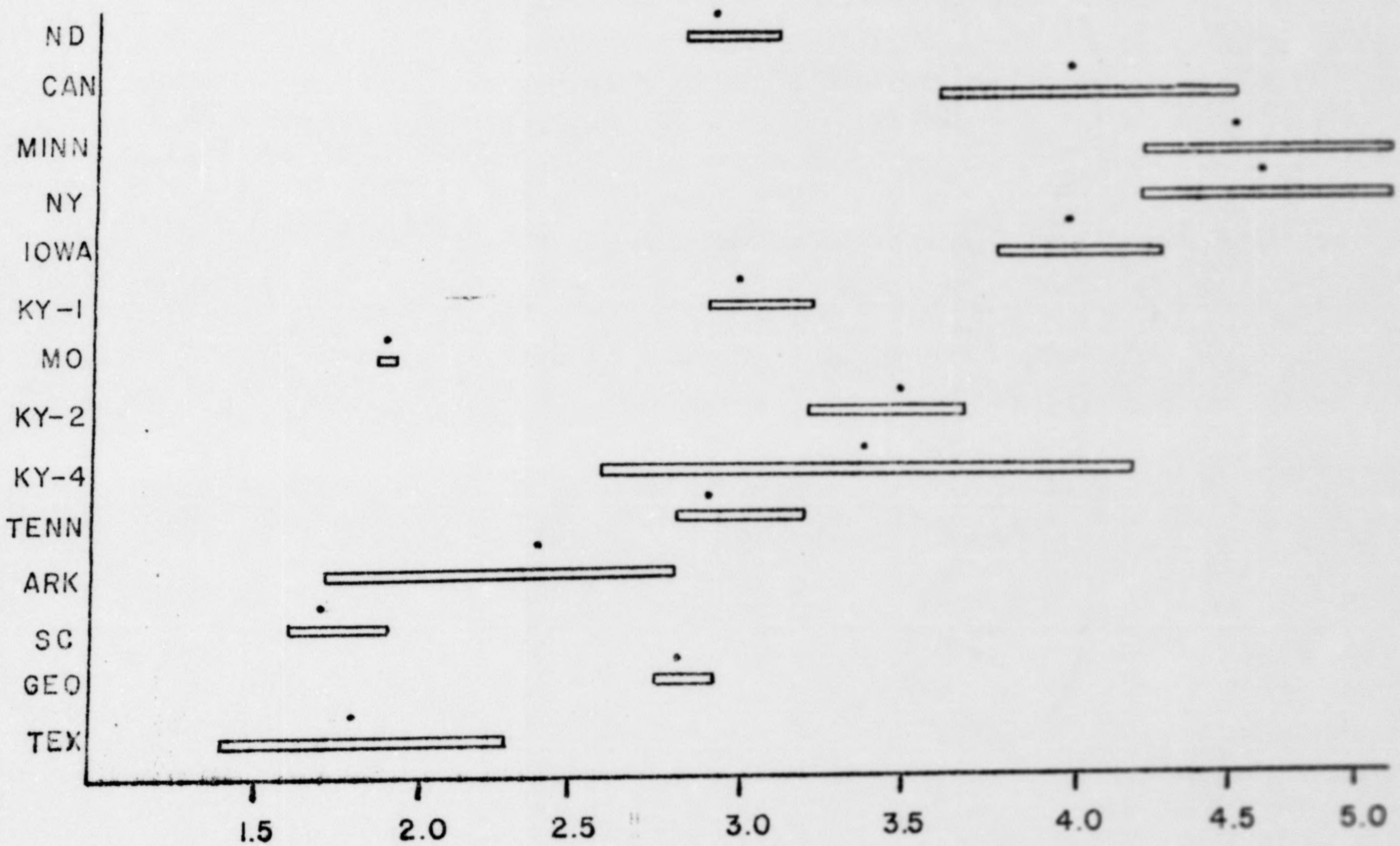

ND
CAN
MINN
NY
IOWA
KY-1
MO
KY-2
KY-4
TENN
ARK
SC
GEO
TEX
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0

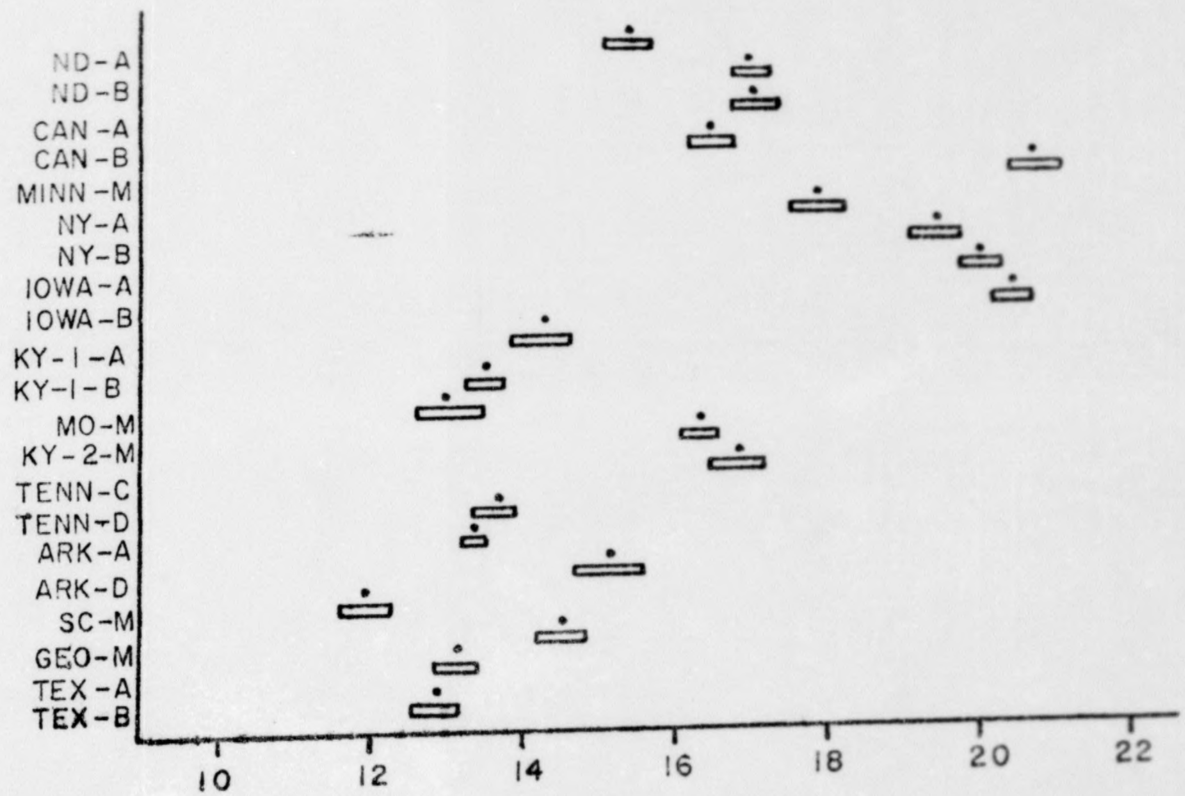
Table 3. Comparisons of fruit lengths, wing lengths, and total lengths of fruits among thirteen populations of *Acer negundo*.

Population	Fruit Length(a)*	Wing Length*	Total Length*
N.D.-A	15.44 ± .22	19.56 ± .32	35.00 ± .44
N.D.-B	17.00 ± .22	18.92 ± .42	35.92 ± .46
Can.-A	17.12 ± .30	13.94 ± .36	31.06 ± .48
Can.-B	16.54 ± .30	17.28 ± .46	33.80 ± .66
Minn.-M	20.76 ± .36	24.36 ± .64	45.16 ± .84
N.Y.-A	17.82 ± .34	17.71 ± .50	35.58 ± .58
N.Y.-B	19.44 ± .32	20.16 ± .66	39.58 ± .36
Iowa-A	20.04 ± .22	19.44 ± .38	39.48 ± .27
Iowa-B	20.50 ± .30	16.42 ± .44	36.92 ± .56
Ky.-1-A	14.26 ± .34	16.42 ± .64	30.68 ± .93
Ky.-1-B	13.46 ± .26	19.44 ± .48	32.88 ± .62
Mo.-M	13.04 ± .44	16.26 ± .44	30.20 ± .58
Ky.-2-M	16.38 ± .24	17.66 ± .36	34.04 ± .48
Tenn.-2-C	16.86 ± .38	15.26 ± .64	32.02 ± .92
Tenn.-2-D	13.66 ± .22	16.30 ± .46	29.96 ± .52
Ark.-A	13.32 ± .16	15.76 ± .34	29.08 ± .40
Ark.-D	15.18 ± .44	16.92 ± .52	32.10 ± .66
S.C.-M	11.90 ± .28	18.28 ± .56	30.18 ± .66
Geo.-M	14.50 ± .22	18.52 ± .32	33.04 ± .36
Tex.-A	13.14 ± .26	15.74 ± .42	28.88 ± .60
Tex.-B	12.80 ± .26	16.00 ± .40	28.80 ± .54

(a) length in mm.

* $\bar{X} \pm t_{(05)} S_{\bar{X}}$

Figure 3. Comparison of the fruit length (exclusive of the wing) among thirteen populations of *Acer negundo*. Mean (circle) and confidence interval (bar) at 0.05.


ND-A
ND-B
CAN-A
CAN-B
MINN-M
NY-A
NY-B
IOWA-A
IOWA-B
KY-1-A
KY-1-B
MO-M
KY-2-M
TENN-C
TENN-D
ARK-A
ARK-D
SC-M
GEO-M
TEX-A
TEX-B
10
12
14
16
18
20
22

at the 0.05 level from Kentucky-2-W, Tennessee, and Arkansas populations. The populations from Kentucky-2 to Texas, with the exception of South Carolina, showed considerable overlapping. South Carolina was the only population tested which significantly differed in fruit length from the other populations.

Comparisons of the wing lengths showed slight increases in length with increases in latitude (Table 3; Figure 4). Texas and Tennessee-2 were the only two populations which lacked intrapopulational variation. Minnesota, the longest-wing material tested, and Canada-C, the shortest-wing material tested, differed from all other populations at the 0.05 level. The remaining populations demonstrated interpopulational variation with degrees of overlapping.

Comparisons of the total fruit length, showed increases with increases in latitude (Table 3; Figure 5). Only the Texas population failed to display intrapopulational differences in total length at the 0.05 level. North Dakota, New York, Iowa, and Minnesota differed significantly from the other populations tested. Minnesota had the longest fruits and differed significantly from all other populations at the 0.05 level. Although the Canadian population was one of the more northern populations tested, fruit lengths ranked with Kentucky and Tennessee populations.

<u>Caloric Value</u>. In seventy-six calorie determinations, there were no significant intra-or interpopulational dif-

Figure 4. Comparisons of the wing lengths among thirteen populations of <u>Acer negundo</u>. Mean (circle) and confidence interval (bar) at 0.05

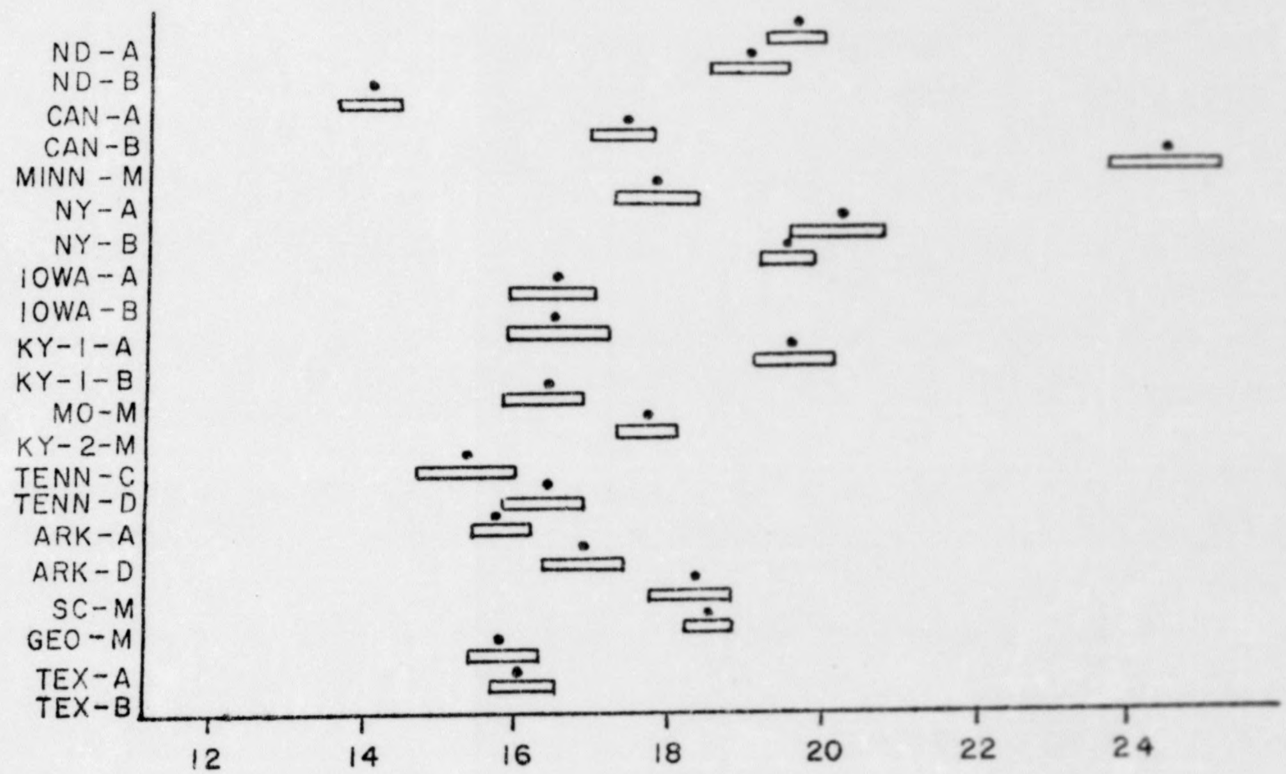
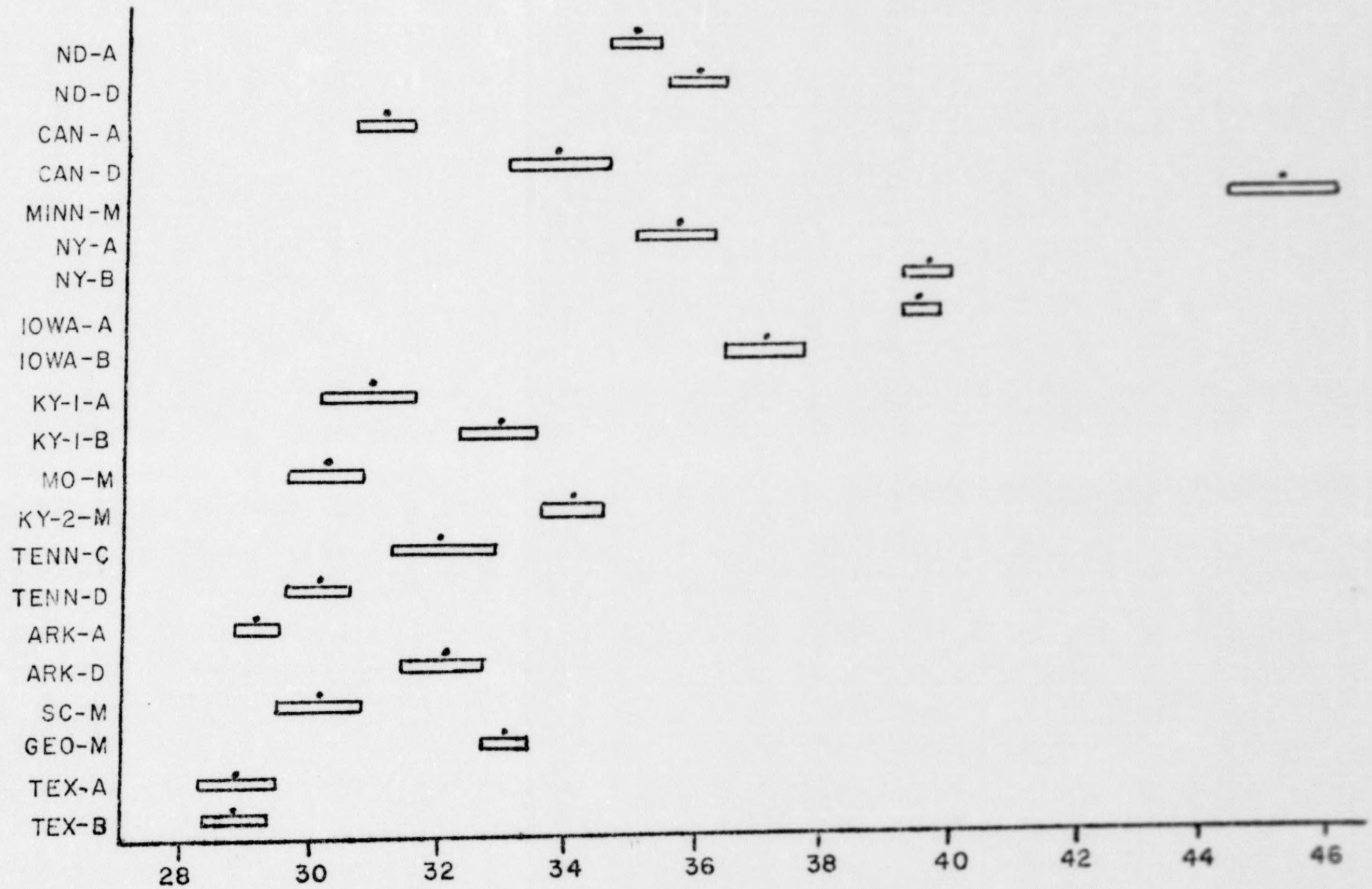

ND-A
ND-B
CAN-A
CAN-B
MINN-M
NY-A
NY-B
IOWA-A
IOWA-B
KY-1-A
KY-1-B
MO-M
KY-2-M
TENN-C
TENN-D
ARK-A
ARK-D
SC-M
GEO-M
TEX-A
TEX-B
12
14
16
18
20
22
24

Figure 5. Comparisons of total fruit lengths among thirteen populations of *Acer negundo*. Mean (circle) and confidence interval (bar) at 0.05.



ND-A
ND-D
CAN-A
CAN-D
MINN-M
NY-A
NY-B
IOWA-A
IOWA-B
KY-I-A
KY-I-B
MO-M
KY-2-M
TENN-C
TENN-D
ARK-A
ARK-D
SC-M
GEO-M
TEX-A
TEX-B
28
30
32
34
36
38
40
42
44
46

ferences as to caloric value per gram (Table 2). The average value for all populations tested was 5,092 calories per gram with a standard deviation of 165 calories. The coefficient of variation among the fourteen populations tested was 3.2%.

Data presented in Figure 6 represents a combination of the average weight per fruit and calories per gram for each population tested. Due to the heavier weight of the fruits from the northern provinces, an higher energy content per fruit is indicated with increases with latitude.

Germination Studies

Stratification Tests. The stratification test showed remarkable variation in seed germination among trees of the same population as well as variation among populations. From the percent germination which occurred during cold stratification, it can be noted that the seeds of the northern provinces germinate early during stratification. Although variation in seed germination among trees of the same population was evident (Table 4), combining the germination results of seed trees of each population indicated a clearer pattern of germination differences (Table 5). Within six weeks of the stratification period, the Kentucky material began to germinate under the colder temperature. Not until the end of the stratification period (10-12 weeks) did the southern populations (Tennessee-2, South Carolina, Georgia and Texas) germinate at the 4-5 C condition.

Figure 6. Estimated calorie value per fruit among thirteen populations of *Acer negundo*.

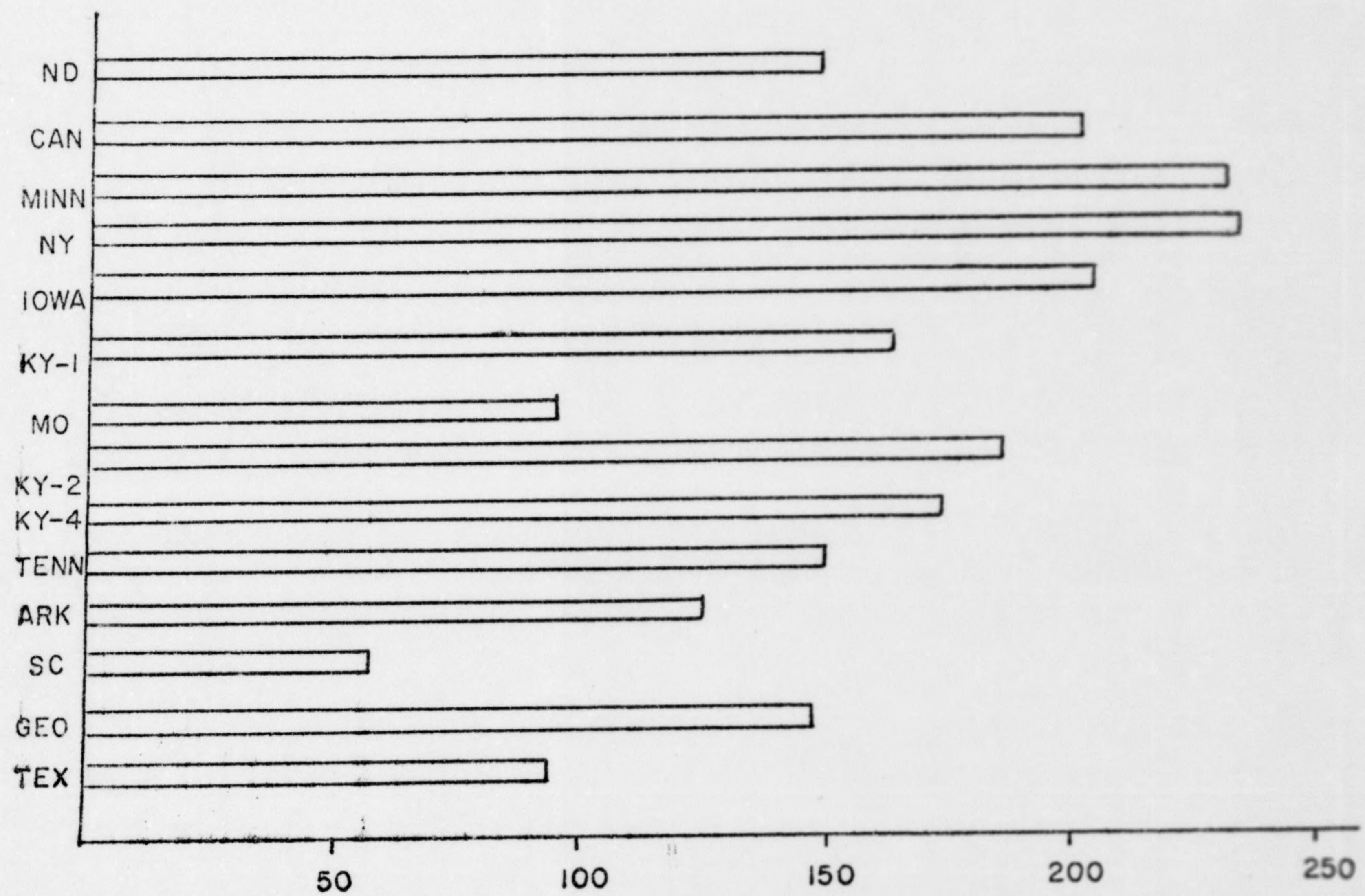

ND
CAN
MINN
NY
IOWA
KY-1
MO
KY-2
KY-4
TENN
ARK
SC
GEO
TEX
50
100
150
200
250

Table 4. Comparison of *Acer negundo* seed germination, by individual tree sources, in response to length of stratification (4-5C).

Tree	Stratification Period									
	4 weeks		6 weeks		8 weeks		10 weeks		12 weeks	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
N.D.-A	4	36	12	26	22	24	16	26	0	0
N.D.-B	0	0	0	2	0	12	0	4	10	14
N.D.-C*	0	2	0	8	0	10	0	8	0	28
N.D.-D*	0	12	0	6	0	18	0	30	2	14
Can.-A	2	4	0	6	0	0	16	22	36	58
Can.-B	10	22	8	8	12	22	14	26	18	38
Can.-C*	0	14	0	18	0	4	4	10	30	32
Can.-D*	0	16	0	10	0	10	10	10	16	40
Minn.-M	0	6	2	14	2	16	4	4	8	8
Minn.-M	0	14	4	20	22	34	24	32	22	24
Minn.-M*	0	22	0	18	0	24	0	30	12	60
Minn.-M*	0	12	0	26	0	6	4	4	4	42
N.Y.-A	0	4	14	22	8	8	0	0	2	2
N.Y.-B	0	18	0	16	26	40	26	34	0	6
N.Y.-B*	0	36	0	38	0	48	6	54	30	40
N.Y.-C*	0	6	2	8	0	8	0	20	6	80
Iowa-A	0	12	0	20	6	14	8	20	16	22
Iowa-B	0	12	0	12	20	48	28	40	8	18
Iowa-B*	0	36	0	34	0	58	4	20	32	78
Iowa-C*	0	48	0	20	0	50	0	50	6	32

Table 4. Cont.

Mich.-M	0	0	4	6	0	0	28	34	0	0
Mich.-M*	0	0	0	0	0	0	6	40	0	28
Ohio-A*	0	16	0	12	0	32	12	40	20	40
Calif.-A*	0	0	0	0	0	0	0	0	0	0
Ky.-3-A	0	0	0	6	0	6	0	14	0	0
Ky.-3-B	0	0	0	10	0	12	2	34	6	20
Ky.-3-C*	0	10	0	8	2	36	10	40	26	80
Ky.-3-D*	0	42	0	50	0	86	18	88	14	58
Ky.-1-A	0	36	8	46	10	40	34	78	20	22
Ky.-1-B	0	24	2	32	18	84	28	48	28	54
Ky.-1-B*	0	10	0	28	10	68	24	72	28	60
Ky.-1-C*	0	30	0	58	2	64	34	72	38	74
Mo.-M	0	2	0	2	0	12	0	14	4	14
Mo.-M*	0	2	0	6	0	14	0	12	0	18
Ky.-2-M	0	50	14	40	28	70	54	86	36	52
Ky.-2-M*	0	34	0	34	6	62	40	80	52	78
Ky.-4-B*	0	2	0	0	0	32	0	24	0	22
Ky.-4-C*	0	4	0	2	0	10	0	46	0	36
Ky.-5-A*	0	2	0	0	0	16	0	52	0	42
Tenn.-1-M*	0	0	0	0	0	4	0	2	0	0
Tenn.-2-A	0	0	0	2	4	20	0	36	4	22
Tenn.-2-B	0	0	0	6	0	14	0	20	0	22
Tenn.-2-C*	0	0	0	6	0	8	0	14	0	8
Tenn.-2-D*	0	8	0	24	0	22	2	32	0	38

Table 4. Cont.

Ark.-A*	0	14	0	16	0	38	4	14	12	34
Ark.-C*	0	4	0	16	0	14	0	14	10	44
S.C.-M	0	0	0	2	0	14	0	4	2	4
S.C.-M*	0	0	0	0	0	0	0	10	0	4
Geo.-M	0	0	0	2	0	2	4	14	0	0
Geo.-M	0	0	0	2	0	0	0	0	8	22
Geo.-M*	0	6	0	6	0	12	0	8	0	22
Geo.-M*	0	4	0	8	0	2	0	4	0	8
Tex.-B	0	0	0	6	0	22	2	16	4	18
Tex.-B*	0	8	0	26	0	22	0	12	0	16
Tex.-D*	0	2	0	18	0	16	0	18	6	34
Tex.-D	0	4	0	10	0	32	6	14	6	34

(1) Percent germination at 4-5C.

(2) Total percent germination after two weeks at 22C, 12-hr day.

* Replication number two.

Table 5. Comparisons of Acer negundo seed germination, by population, in response to length of stratification.

Population	Stratification Period: 4 weeks (1)	4 weeks (2)	6 weeks (1)	6 weeks (2)	8 weeks (1)	8 weeks (2)	10 weeks (1)	10 weeks (2)	12 weeks (1)	12 weeks (2)
N.D.	1.0	11.5	3.0	7.5	5.5	10.5	4.0	13.0	3.0	11.0
Can.	3.0	11.0	2.0	8.5	3.0	6.0	11.0	6.5	25.0	17.0
Minn.	0	13.5	1.5	18.0	6.0	14.0	8.0	9.5	11.5	22.0
N.Y.	0	15.5	4.0	17.0	8.5	17.5	8.0	14.0	9.5	22.5
Iowa	0	27.0	0	21.5	6.5	36.0	15.0	17.5	15.5	22.0
Mich.	0	0	2.0	1.0	0	0	17.0	20.0	0	14.0
Ohio	0	16.0	0	12.0	0	16.0	12.0	34.0	20.0	20.0
Calif.	0	0	0	0	0	0	0	0	0	0
Ky.-3	0	13.0	0	18.5	1.0	34.5	12.5	36.5	11.5	28.0
Ky.-1	0	25.0	2.5	38.5	10.0	39.0	30.0	38.5	28.5	24.0
Mo.	0	2.0	0	4.0	0	9.5	0	13.0	2.0	1[illegible].0
Ky.-2	0	42.0	7.0	30.0	17.0	45.0	47.0	36.0	44.0	21.0
Ky.-4	0	3.0	0	1.0	0	21.0	0	35.0	0	29.0
Ky.-5	0	2.0	0	0	0	16.0	0	52.0	0	42.0

Table 5. Cont.

Tenn.-1	0	0	0	0	0	4.0	0	2.0	0	0
Tenn.-2	0	0	0	9.5	1.0	15.0	0.5	25.0	1.0	21.5
Ark.	0	9.0	0	16.0	0	26.0	2.0	12.0	11.0	28.0
S.C.	0	0	0	1.0	0	2.0	0	2.0	1.0	3.0
Geo.	0	2.5	0	4.5	0	4.0	1.0	5.5	2.0	11.0
Tex.	0	2.5	0	15.0	0	23.0	2.0	13.0	4.0	21.5

(1) Percent germination during stratification (4-5C).

(2) Percent germination at 22C, 12-hr day.

In a similar trend germination during stratification increases to an extent in the North Dakota to Kentucky populations in that the germination at the colder temperature is equal to, or greater than, the germination at the warmer temperature (Table 5).

Observing the total germination percent (Table 4, Column 2) it can be noted that some of the more northern populations (New York to Iowa) and intermediate latitudinal populations (Kentucky) reach or exceed the reported germination capacity of 33% for <u>Acer negundo</u> (Vines, 1960) under four to six weeks of stratification. Southern populations (Tennessee-2, Georgia, and Texas) reached higher germination ranges only after eight to twelve weeks of stratification.

<u>Temperature Preference</u>. The temperature preference test did not display any general trend or segregation of the populations at the three temperature regimes used. Germination under the alternating temperatures of 30 C day and 18 C night appeared only to stimulate germination in the North Dakota and New York populations (Table 6). Germination appeared more extensive in the 11 C temperature conditions than at the other two temperature regimes. Here, as in the stratification test, there was extensive germination of some of the more northern populations during the period of stratification (Table 6, Column 1).

Inhibitor Studies

<u>Pericarp</u>. Study A: In the non-stratified material,

Table 6. Comparison of *Acer negundo* seed germination, by ten populations, in response to three different temperature programs.

Population	Percent Germination					
	11 C		22 C		30-18 C	
	(1)	(2)	(1)	(2)	(1)	(2)
N.D.-C	10	18	2	2	4	26
N.D.-D	2	0	4	2	4	4
Can.-C	0	2	38	14	0	0
Can.-D	46	28	26	24	52	10
Minn.-M	0	6	0	2	6	2
Minn.-N	4	4	0	2	4	0
N.Y.-B	26	18	30	6	10	2
N.Y.-C	22	40	14	14	12	68
Iowa-B	22	22	4	10	0	2
Iowa-C	14	32	8	10	0	12
Mo.-M	0	0	0	0	0	0
Ky.-5-A	8	36	0	4	0	8
Tenn.-1-M	0	0	0	16	0	0
S.C.-M	0	0	0	0	0	0
Geo.-M	18	34	2	6	0	0

(1) Percent germination during stratification.

(2) Percent germination at temperature condition.

removal of the pericarp induced germination up to 48% (Iowa-B) in twelve days. Whereas, in the material with the pericarp intact, no germination was recorded in the twelve day period (Table 7). Germination proceeded at a faster rate in the depericarped Iowa and Kentucky material (mean daily germination rate at 4.5% and 1.2%, respectively) than in the Tennessee and Georgia material (mean daily germination rate 0.66% and 0.26%, respectively).

In the stratified material, germination occurred in both the depericarped and intact pericarp group. The depericarped material demonstrated an higher germination percent and signs of germination (i.e., extrusion of the radicle through the seed coat) earlier than the seed material with the pericarp intact.

Study B: Removal of the pericarp stimulated germination whether the seed material was placed under photoperiod or dark conditions (Table 8). An aqueous extract of the pericarp did not seem to inhibit the germination of depericarped material, but rather stimulated the germination of boxelder seed (Table 8).

Inhibitory Effects of Aqueous Fruit Extract. Study A: An aqueous fruit extract apparently inhibited the germination of both radish and lettuce seeds (Table 9). The inhibition decreased with time and by the third day of germination was eliminated. Visual observation showed longer radicles and well developed root hair regions on the radish and lettuce control which were absent or highly reduced on

Table 7. Effect of removal of the pericarp in non-stratified and stratified *Acer negundo* populations.

Population	Non-stratified Material(a)		Stratified Material(b)	
	Pericarp Removed	Pericarp Intact	Pericarp Removed	Pericarp Intact
Iowa-A	6	0	92	18
Iowa-B	48	0	--	--
Ky-2-M	14	0	90	50
Ky-2-M	26	0	--	--
Tenn-2-A	8	0	72	22
Tenn-2-B	0	0	68	0
Geo-M	2	0	70	14
Geo-M	13	0	--	--

(a) Percent germination at 22C 12-hr day, 12th day recorded.

(b) Material stratified for four weeks at 4C, 12th day germination count.

Table 8. Comparison of light and dark response of seed and fruit material of *Acer negundo*.

Population	Light(a)			Dark(b)	
	Seed	Seed with pericarp extract	Fruit	Seed	Fruit
Geo-M	13	20	0	10	0
Geo-M	2	--	0	--	-
Ky-2-M	26	40	0	30	0
Ky-2-M	14	--	0	--	-

(a) 22C 12-hr-day, percent germination on the 12th day.

(b) 22C dark, percent germination of the 11th day.

Table 9. Comparison of inhibitor effect of fruit and wing extracts from *Acer negundo* on radish and lettuce seed.[1]

Replicates	Fruit Extract						Wing Extract					
	Radish			Lettuce			Radish			Lettuce		
Geo-M	10	40	90	8	26	92	10	98	100	2	78	94
Geo-M	8	44	90	2	2	92	6	92	98	0	90	100
Geo-M	6	66	94	0	4	62	14	96	100	8	84	100
Control	58	100	100	100	100	100	58	100	100	100	100	100
Days	1	2	3	1	2	3	1	2	3	1	2	3

1. Numbers represent percent of germination of 50 seeds.

the seeds treated with the extract.

The aqueous extract of the wing material displayed inhibitory effects. These effects diminished by the second day of germination. As in the entire fruit extract tests, there were visual differences between length and root hair development of the radicles of the controls and those treated with the wing extract.

Study B: Aqueous extracts of pericarps alone, seeds alone, and fruits inhibited germination of lettuce seed (Table 10). The seed extract showed less inhibitory effect than the extract derived from the entire fruit, or pericarp alone. Inhibition of germination decreased by the second day and appeared to be greatly reduced by the third day.

Study C: All the aqueous extracts of the fruit material from the ten populations tested demonstrated some inhibitory effect against the germination of lettuce seed (Table 11). As in the other inhibitor tests, the effect was the greatest on the first day and diminished by the second day. The Kentucky and Iowa populations displayed the least inhibition on the first day of the test, and the Minnesota and Missouri populations displayed the greatest inhibition on the first day. Georgia was the only population which inhibited germination of the lettuce seed on the second day of the test. Overall, the data presented in Table 11 gives the impression of a normal curve with the extreme north and south populations used in the test demon-

Table 10. Inhibitor effect on lettuce seed of extracts from fruit, seed, and pericarp of a Minnesota population of *Acer negundo*.

Materials	Days 1	Days 2
Pericarp	0	70
Seed	42	96
Fruit	0	72
Control	94	96
Pericarp	8	--
Seed	86	--
Control	96	--

Table 11. Comparison of inhibitor extracted from fruit of ten populations of *Acer negundo* on lettuce seed.

Population	Percent Germination	
	Days 1	2
Can.-C	4	90
Minn.-M	0	96
Iowa-B	16	98
Ky.-3-D	10	100
Ky.-1-B	24	96
Mo.-M	0	84
Ky.-2-M	24	100
Ky.-4-A	10	84
Tex.-D	6	98
Geo.-M	6	66
Control	84	98

strating the most inhibition, with the exception of the Missouri material.

Solubility Test. Study A: Water extract of the fruit material inhibited the germination of lettuce seed which did not diminish until the fourth day of germination. There was no inhibitory effect noted in the ether wash of the water extract (Table 12).

Study B: The water extract of the material demonstrated inhibition of germination of lettuce seed while the chloroform wash of the water extract displayed no inhibitory effect. Inhibition by the water extract diminished by the third day of germination (Table 13).

Study C: The methanol extract of the fruit material did not inhibit the germination of lettuce. The ether wash of the methanol extract demonstrated did display inhibition of germination of the lettuce seed (Table 14).

Study D: The ethanol extract displayed inhibition of the germination of lettuce seed (Table 15). This inhibition diminished by the second day of germination. The petroleum ether wash of the ethanol extract did not inhibit the germination of lettuce seed.

Inhibitor-Stratification Test. Study A: The inhibition appeared to decrease with one week of cold stratification in most cases but increased after two weeks with fluctuation through the eight week test period (Table 16). There seemed to be no recognizable trend showing an overall decrease in inhibition with increase in stratification time.

Table 12. Comparison of inhibitor in water extract and ether wash of water extract from fruit of Acer negundo on lettuce seed.

Population	Percent Germination of Lettuce									
	Water Extract After Ether Wash					Ether Wash of Water Extract				
Geo-M	0	0	0	44	64	100	100	100	100	100
Geo-M	0	0	8	88	92	92	96	96	100	100
Minn-M	0	0	8	46	72	100	100	100	100	100
Control	96	98	98	100	100	96	98	98	100	100
Days	1	2	3	4	5	1	2	3	4	5

Table 13. Comparison of inhibitor in water extract and chloroform wash of water extract from fruit of *Acer negundo* on lettuce seed.

Population	Percent Germination of Lettuce: Water Extract After Chloroform Wash					Percent Germination of Lettuce: Chloroform Wash of Water Extract				
Geo-M	0	4	4	14	44	96	100	100	100	100
Geo-M	0	4	10	42	74	82	100	100	100	100
Minn-M	0	6	84	100	100	98	98	98	98	98
Control	98	100	100	100	100	98	100	100	100	100
Days	1	2	3	4	5	1	2	3	4	5

Table 14. Comparison of inhibitor in methanol extract and ether wash of methanol extract from fruit of *Acer negundo* on lettuce seed.

Population	Percent Germination of Lettuce					
	Methanol Extract After Ether Wash			Ether Wash of Methanol Extract		
Geo-M	98	100	100	70	98	100
Geo-M	94	100	100	98	100	100
Control	98	100	100	98	100	100
Days	1	2	3	1	2	3

Table 15. Comparison of inhibitor in ethanol extract and petroleum ether wash from fruit of *Acer negundo* on lettuce seed.

Population	Percent Germination of Lettuce: Ethanol Extract After Petroleum Ether Wash		Petroleum Ether Wash of Ethanol Extract	
Geo-M	74	100	98	100
Geo-M	88	98	98	98
Minn-M	6	96	96	98
Control	96	100	98	100
Days	1	2	1	2

Table 16. Comparison of inhibitor from fruit of five populations of *Acer negundo* extracted during eight weeks of stratification.

Population	Stratification Period (weeks) 0	1	2	3	4	5	6	7	8
N.Y.-A	0[a]	94	32	67	33	42	98	12	50
N.Y.-B	8	90	62	11	37	13	34	67	26
Iowa-A	14	14	38	30	17	56	34	55	20
Iowa-C	21	90	2	30	6	19	20	51	26
Ky.-1-A	44	92	55	44	23	71	75	31	16
Ky.-1-C	25	98	4	7	15	35	24	2	76
Tenn.-2-A	10	2	21	0	33	54	43	63	52
Tenn.-2-B	4	44	26	11	83	6	76	76	22
Geo.-M	0	20	4	11	35	54	37	14	26

(a) Germination of lettuce expressed as percent of control on the first day of germination.

Study B: Testing for any inhibitory effect contained in elutants from filter paper on which seeds had been stratified for periods of one to eight weeks was generally negative. Taking filter paper elutant from nine fruit lots of Georgia to New York origin which were stratified one through eight weeks provided sixty-three repeated tests for inhibition of lettuce seed. In only three of these tests were there any apparent inhibition of the paper elutants on the bioassay.

DISCUSSION AND CONCLUSIONS

As assumed, because of the wide distribution of *Acer negundo* L., this woody species does exhibit some populational variation. Not all the differences observed demonstrate latitudinal variation but with further inquiries may show local adaptations.

Comparisons of the mean weight per hundred fruits of the fourteen populations sampled demonstrate a definite cline with fruits originating from the northern habitat being heavier. Similar results for *Amaranthus rextroflexus* L. have been demonstrated by McWilliams, et al. (1968) using seed material obtained from plants grown under uniform conditions. McWilliams and co-workers omitted several populations from their discussion of seed weight "because elevation differences were large in these collection sites, and considerable variation might be expected due to this difference alone." This statement may give partial explanation for the variance from the trend of *Acer negundo* by the North Dakota and Missouri populations. The elevation of the North Dakota site was 1,600 feet and that of Missouri was 1,300 feet. However, the Iowa population at an elevation of 1,000 feet fit the general trend of increase in fruit weight with increase in latitude. If the stimulus for heavier fruit of the northern habitats is a reduced growing season, it would appear that populations at higher altitudes would also exhibit greater fruit weight. Sharik (personal communication, 1970) indicates similar patterns of smaller seeds of Betula at high altitudes in North Carolina. At present, there appears to be limited explanation for variation in seed size when correlated to altitude.

Since fruit weight is somewhat of a quantitative character, it can be assumed that partial weight differences may be due to preconditioning of the material by the various environmental conditions at the collection sites. Harper (1961) has pointed out, however, that mean seed weight has been considered one of the least plastic properties of plants. As the fruit weight varied among the populations so did the size (length). The size of the fruit is smaller in the southern latitudes and increases in size with increase in latitude. A similar trend has been noted in populations of Phoradendron ranging from North Texas to Mexico, with larger fruits occurring in northern populations decreasing in size to the Mexico populations (May, 1969). The total length of the fruits in the current study also demonstrated a latitudinal cline with the smallest material occurring at the lower latitude.

Morley and Katznelson (1965) have pointed out that selection for increased seed size may be accompanied by a disadvantage in dispersability. Since Acer negundo is a meteoranemochorous (winged flyer) plant (van der Pijl, 1969) one would expect a decrease in the distance that the diaspore (dispersal unit) could be displaced with the increasing weight. In <u>Acer negundo</u>, however, there appears to be selection for longer wing length as well as heavier fruit. Those populations which exhibited heavier fruit material also exhibited longer wings resulting in a latitudinal cline with the lighter, shorter winged material in the southern populations and with both factors increasing with increasing latitude. Some populations varied from this general trend (as Canada-A) showing a physical hinderance on the dispersal mechanism (the samara). Such an hinderance could affect the migration, colonization, and seeding potential of the population.

As suggested by Long (1934) and stimulated by the work of Lindeman (1942), workers have been using the bomb calorimeter to study productivity and to compare caloric values of plant parts from different geographical locations and habitats or between new and old field ecosystems (Golley and Gentry, 1966; Golley, 1969). The fourteen populations ranging from North Dakota to Texas used in the calorie determinations did not appear to vary from one another when considering calories per gram. Similar results were reported by Johnson and Robel (1968) for nine species of plants from four distinct range sites. In their work they detected no differences between mean energy content of seeds from the four sites, but they did find intraspecific differences between means of energy content of seeds from different locations within the same range sites. Golley (1969) has stated that "previous studies showed that fruits (seeds included) and roots have greater energy values than other vegetation components due to energy stored in these organs." Golley also concludes that there is a gradient in energy content of vegetation from the equatorial to higher latitudes or altitudes; however, within a geographical region, the energy values may vary from site to site or with the portion of the vegetation being sampled. In certain situations where storage of high energy reserves may be of value to the vegetation, the latitudinal gradient may be obscured. This may be the case for Acer negundo. Since the seed material is a storage tissue for the development of a new plant, the trend, if present for entire plants, could be obscured by the selective analysis of seeds. However, since there is also a weight difference among these populations, there could be a total calorie difference. Assuming that all trees involved in the populations studies produced the same mean quantity of seed material, the northern populations, based on total biomass and calorie content, may be more productive. This indicates the need for both knowledge of total biomass and calorie content before any two ecosystems or populations can be compared with any degree of accuracy.

Larger seeds may have competitive advantage in that the larger food reserve can allow for faster germination and seedling establishment in a shorter period of time (Black, 1958). As shown in Figure seven (7) the northern populations, due to increased weight, possess higher caloric content per fruit. This higher calorie content per fruit may allow them to become established within the shorter growing season. However, this reasoning assumes that all the determined calorie content can be utilized by the young embryo neglecting the energy requirements of the northern populations for a longer period of dormancy.

Clinal responses to stratification of seeds from different sources have been reported for woody species. Fowler and Dwight (1964) and Mergen (1963) reported that white pine showed clinal response related to both latitude and mean January temperature. Wilcox (1968) and Winstead (1968) reported clinal response to latitude in seed germination by Liquidambar styraciflura L. Wilcox concluded that seeds from more northern sources required a longer period of stratification in order to prevent early germination and subsequent killing of seedlings by late frosts. Acer negundo does not demonstrate a clear clinal stratification response to latitude but exhibits separation into two major geographical groups. Both stratification tests demonstrate the ability of the populations from Kentucky northward to germinate during cold storage. It also appears that the southern populations (Tennessee southward) require longer stratification periods to induce greater germination percentages. This trend observed in Acer negundo is contrary to the studies cited above and may reflect factor(s) other than, or in combination with, length of stratification period as the regulator of germination.

Germination of material during cold storage has been reported for black and white spruce, mountain, silver, and sugar maple, basswood, and beech (MacArthur and Fraser, 1963). These tree species germinated in the cold after prolonged stratification up to one year or longer. The length of stratification used in the stratification test was not considered excessive and is the recommended period of time of cold storage for Acer negundo (Anonymous, 1961).

The temperature preference may indicate segregation of the populations into two groups in that the northern populations appeared to germinate more readily at the higher or alternating temperatures. All the populations appeared to germinate equally well at the lower, 11 C temperature. Vickery (1967) has pointed out that extreme temperature ranges segregate populations of Mimulus. To determine if Acer negundo does exhibit differences in germination at different temperatures, more temperature ranges with extreme temperatures need to be tested.

Germination studies with Acer negundo are complicated by the germination of some of the populations in cold storage and differences in the quality of the fruit collections. It is interesting to note that May (1969) and McWilliams, et al. (1968) observed more complete germination in the northern populations as is the case in Acer negundo. The significance of these observations remains to be determined.

As indicated by Fowler and Dwight (1964), populations of seed plants from higher latitudes of northern United States and Canada may not be exposed to fluctuating temperatures in the spring as is common between latitudes of 33-37. In more northern habitats, once the spring temperatures rise above freezing, the natural habitat is not subject to wide temperature fluctuations and thus is not exposed to a selective factor that would require long periods of dormancy at low temperature.

The mechanism(s) which inhibits the germination of Acer negundo and the apparent need of cold stratification to induce germination is obscure. Nikolayeva (1951) reported that dormancy in Acer negundo was caused not by the dormancy of the living tissues, but by the existence of compact dead covers, seed skin and pericarp. Injury or the removal of the pericarp induces normal germination. Vines (1960) suggested that dormancy can be removed by leaching with cold running water. Irving (1968) concluded that dormancy in Acer negundo is due to an inhibitor(s) in the seed coat (meaning pericarp) and or wing and is not the result of a dormant embryo as previously reported.

The present study has shown that removal of the pericarp does induce germination under light or dark conditions. Irving (1968) reported the same results but stated that germination of the depericarped material was one-hundred percent. None of the populations tested in the current study displayed this trend. Only after stratification of the depericarped material did the germination improve indicating some embryonic inhibition.

Aqueous extracts of the seed, wing, and pericarp (including the wing) inhibit germination of lettuce and radish seed. Extracts of excised pericarp and of the wing show an high concentration of inhibitor(s) in these materials. Extracts of the seed show only a slight concentration of inhibitor(s) as determined by bioassays. It has been previously reported that an extract of unchilled, dewinged fruits produces inhibition of germination in a lettuce seed test at the same R_f as abscisic acid (ABA) (Irving, 1968). Abscisic acid is somewhat water soluble (Ohkuma, et al., 1963) and has been related to dormancy in Fraxinus and apple seeds (Sondheimer, et al., 1968; Rudnicki, 1969). Abscisic acid has also been isolated from the short day leaves of Acer negundo (Irving, 1969). It is probable, therefore, that abscisic acid is the inhibitor involved as this work clearly indicates the inhibitory factor is water soluble. Irving, however does not mention inhibition of lettuce seed by an extract of the embryo tissues. The present study indicates the presence of inhibitory substance(s) in the embryo which supports the earlier statement of Vines (1960).

Inhibitor(s) extracted from the seed coat, pericarp, and glume of plants other than boxelder has been shown to restrict the germination of the seeds from which the material has been removed (Witcombe, 1969; Wurzburger, et al., 1969; Sondheimer, 1968). Although the extract of the pericarp did not inhibit germination of Acer negundo, the lack of allopathy may be the result of too low a concentration of the inhibitor(s). The low concentration of the inhibitor(s) may be responsible for the apparent stimulation of germination of boxelder seed as shown in other plants (Mayer and Poljakoff-Mayber, 1963). Further work is necessary to determine if the inhibitor(s) in the pericarp are allopathic.

The extract of the fruit material from ten populations tends to indicate that there may be a difference in the amount of inhibitor(s) present on a fresh weight basis. This possible difference in amount of inhibitor(s) may give partial explanation for the results of the stratification test. As shown in the inhibitor test, the Kentucky populations as a whole demonstrated less inhibition than the other populations tested. Throughout this study the Kentucky populations as a whole demonstrated remarkable diversity. Other workers have shown the absence or presence of inhibitors as they effect the autoecology of plants (Bell and Amen, 1970; Winstead, 1968). Further work is required to substantiate inhibitor difference and to determine the role of this difference, if present, in the distribution of Acer negundo.

Rudnicki (1969) reported a decrease in the levels of abscisic acid in apple seeds during cold, moist stratification. *Acer negundo* did not show any appreciable decrease in the inhibitor effect during cold, moist stratification. In working with *Fraxinus americana*, Sondheimer, et al. (1968) reported that the levels of abscisic acid in the seed decreased with chilling treatment and that the decrease of the abscisic acid in the pericarp was small during the cold treatment. Sondheimer, et al. (1968) also stated that there is no clear relation between the physiological state of the embryo and the ABA level in the pericarp. They also suggested that since the percentage of ABA lost was significantly greater in the seed than in the pericarp, the loss inside the seed is due to enzymatic action while that in the pericarp is caused primarily by diffusion, and the function of ABA in the pericarp is connected with the regulation of senescence or abscission.

Considering the results of the inhibitor-stratification test reported here, it may be possible that the bioassays were reflecting the amount of inhibitor in the pericarp, which remained fairly constant. If there was a change in the balance of promoters and inhibitors in the seed, the bioassay may not have been sensitive enough to reflect this concentration of inhibitor(s) in the pericarp.

The results of the elutant test lend support to this theory in that no inhibitor was detected on the filter paper. This also could have resulted from too low a concentration for detection by the bioassay or loss of the inhibitor by the method in which the filter paper was manipulated.

Since the pericarp is maternal, all the fruit material of the same parental tree would possess the same genotypic makeup in the pericarp. If this were the case and the pericarp contained the sole inhibiting factor to germination, one would expect considerable homogeneity in germination responses in material from the same tree. This was not observed. The same reasoning was used by Taylorson and McWhorter (1[illegible]) in germination of ecotypes of *Sorghum halepense*. However, if there is dormancy of the embryo, which would be related to its genotype, the dormancy of the seed would be interaction of both the embryo and the pericarp. This would tend to result in the variation noted in the stratification test. The mechanism and role of the pericarp in the dormancy of *Acer negundo* has not been determined. It may be that the pericarp is impermeable to water or gas and the permeability is affected by cold treatment. This study indicates that the pericarp does influence germination but that a cold period is required for stimulation of the embryo.

When considering a problem as disclosed here, it must be kept in mind that there is a lack of knowledge on what influence preconditioning could have on the material used in this study and others. This is one of the inherent errors of a problem of this nature. Selection of one environmental parameter to control germination might be in error, since all are interrelated and a summation of the total environment. The environment of a seed is not the woodlands or forest but consists of a micro-environment in which the physical conditions in close proximity to the seed itself primarily affect the germination process. Future studies should consider the soil, leaf litter, and organisms associated with the seed to determine the additive action for which they might be responsible. It is questionable whether the sterile environment of the germination chamber is representative of natural conditions for seed germination. It is obvious that laboratory conditions only provide part of the answer to the ecology of germination, but they are necessary since field conditions are too complex to fully duplicate.

This study has provided preliminary insights into possible mechanisms of adaptation for Acer negundo. The data reported here indicate that Acer negundo is not a genetically uniform plant and suggests further study on the regulation of germination and its effect upon the geographical distribution of this woody species. While the significance of heavier fruits with more total caloric value remains unclear, the energy determinations do provide support for the rather recent hypothesis that more northern, temperate plants exhibit greater productivity than southern, temperate or tropical populations of the same species. Such data may become of increasing interest in future utilizations of existing natural ecosystems. Ecotypic variation in seed germination was not striking in a clinal sense but did exhibit a broad pattern of possible adaptations to cooler temperatures in the more northern populations tested. The presence of a water soluble germination inhibitor has been documented which confirms other unpublished reports of an inhibitor in this species.

SUMMARY

1. Comparisons of the mean weight per hundred fruits of fourteen populations demonstrate a definite cline, seed originating from the northern habitats being heavier.

2. The fruit length, wing length, and total length of the fruit material from thirteen populations increases with increase in latitude.

3. The average caloric value for all populations tested was 5,092 $\pm$ 165 calories per gram. Due to the heavier weight of the fruit from the northern provinces, an higher energy content per fruit is indicated with increase in latitude.

4. Stratification and germination tests separate the populations into two large geographical groups with the northern populations germinating at the colder (4-5 C) temperatures and the southern populations requiring longer stratification periods and warmer germination temperatures.

5. Removal of the pericarp induces germination. Stratification of the depericarped material increases the germination capacity.

6. There is an highly water soluble inhibitor (probably abscisic acid) in both the pericarp and embryo tissues.

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VITA

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