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The Role Of Genetic And Phenotypic Variation In The Colonization Biology Of The Weedy Nightshade, Solanum Ptycanthum Dun

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The role of genetic and phenotypic variation in the colonization biology of the weedy nightshade, *Solanum ptycanthum* Dun.

by

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ABSTRACT

Solanum ptycanthum (eastern black nightshade) is a serious weed of tomato and soybean crops at the northern margin of its distribution in southwestern Ontario. Evidence for direct immigration, albeit at low, sporadic frequencies, of nightshade into these agrestal habitats occurs via tomato transplants imported from Georgia and North Carolina. Northern ruderal populations are also common in a variety of natural habitats. Outcrossing rates in northern and southern genotypes were compared in simulated populations grown in northern agricultural habitats. Greenhouse experiments were used to examine levels of genetic variation and phenotypic plasticity in life history traits among ruderal, agrestal and southern populations in response to abiotic factors which would be experienced when invading northern agroecosystems.

Genotypes from recently colonized northern populations (both agrestal and ruderal) had lower outcrossing rates (<3%) than those originating from the south (3-17%).

Northern agrestal populations were not less genetically variable, and did not express greater phenotypic plasticity than northern ruderal or southern agrestal populations under a wide range of greenhouse conditions. Germination speed was greater, and the number of degree days to reach 50% germination was less in ruderal families, suggesting that ruderal populations would emerge earlier than agrestal

populations. The germination response to temperature was similar between northern and southern agrestal populations, suggesting that these populations would emerge synchronously.

Ruderal and northern agrestal populations were equally tolerant to low doses of metribuzin, while populations originating from Georgia were extremely susceptible to both levels of metribuzin tested. North Carolina seedlings were as tolerant as northern agrestal populations. Northern agrestal populations were more tolerant than ruderal populations at the highest dose of metribuzin.

There was no divergence in the phenotype expressed by ruderal and northern agrestal individuals in response to variation in nutrients. There was no evidence for selection for increased "yield" response in agrestals grown under high nutrient conditions. However, delayed reproduction of southern compared to northern plants may limit the production of viable seed in northern short-season crops. Developmental pattern and age to first reproduction were canalized within southern populations, suggesting lack of sufficient genetic variability to respond to selection encountered during colonization of northern agroecosystems.

To Gertrude and the late J. Phillip Hermanutz, whose strength in adversity made me realize my inner potential as a person.

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1.1 General introduction

The ability of many species to aggressively and successfully invade novel territories and habitats has long been of interest to biologists (Baker and Stebbins 1965; Brown and Marshall 1981). Much of the research effort has been directed towards economically important, and obviously successful colonizing species such as weeds, whose long- and short-distance migrations have been well documented in the past. Baker (1965, 1974) noted a suite of life history traits which characterized an "ideal" weedy syndrome: non-specific germination requirements, rapid development to flowering, high seed productivity, autogamy, well developed seed dispersal, and tolerance of a wide range of environmental conditions (phenotypic plasticity). These traits are consistent with the r-selected life history (MacArthur and Wilson 1967), or with the "ruderal" strategy of Grime (1979). As pointed out by Barrett (1982, 1988), "weeds" are a very heterogeneous group originating from diverse taxonomic backgrounds, with a wide range of reproductive strategies and as such do not necessarily conform to these "ideals".

In the past 10 years there have been numerous reviews which have characterized the genetic attributes associated with the colonizing ability of weeds (Brown and Marshall 1981; Barrett 1982, 1983; Oka 1983; Barrett and Richardson

1986; Barrett 1988; Warwick 1990a). These studies have elucidated the following common genetic features of weed species: 1) reproduction via autogamy or asexual means, 2) genetically depauperate populations, 3) reduced number of genotypes resulting from multilocus associations, 4) substantial interpopulation differentiation, 5) high levels of phenotypic plasticity, and 6) polyploidy. The realized amounts and organization of genetic variation in weed populations are the outcome of several potentially interacting historical and selective factors commonly associated with newly established populations (Clegg and Brown 1983). A primarily autogamous mating system promotes the non-random association of loci, resulting in a decreased number of multilocus genotypes, and reduced neighbourhood sizes (Allard, Jain and Workman 1968; Hamrick and Godt 1990). In addition, new colonies are usually founded by a limited number of immigrants from the source population, further constraining the number of genotypes. Together with drift after establishment, selfing and genetic bottlenecks can potentially result in decreased genetic variability (Barrett 1988; Warwick 1990a). Such a reduction in genetic variation (estimated from isozyme data) has been found in colonial relative to source populations of several species (Clegg and Brown 1983). However, significant levels of genetic variation in life history traits (i.e., quantitative traits) have been detected in recently founded weed

populations (e.g., Wu and Jain 1978; Moran, Marshall and Muller 1981; Hume and Cavers 1982; Weaver, Warwick and Dirks 1985; Warwick and Black 1986; Barrett 1988; Blais and Lechowicz 1989; Warwick 1990b). Barrett (1988) suggested that measures of genetic variability based on quantitative polygenic traits are more likely to reflect their direct adaptive value, whereas the unknown nature of the functional significance of isozymes makes evaluation difficult. Warwick (1990b) has also suggested that the incongruity between the two measures of genetic variation may reflect the recent divergence of life history traits in response to natural or human-generated selection, while such response has lagged in neutral isozyme genes.

Compared with outcrossing species, selfers tend to have high levels of interpopulation differentiation and low levels of genetic variation (i.e., based on enzymes) within populations (Hamrick and Godt 1990). In predominantly selfing weedy species, interpopulation differentiation may be a consequence of variable selection pressures associated with the diverse habitats colonized by weeds. Baker (1965, 1974) and Holzner (1982) have differentiated weed types based on habitat: agrestal which are associated with agroecosystems and ruderal which are found in non-agricultural lands, but still dependent on disturbance (e.g., waste places and roadsides). The categories are not mutually exclusive (Barrett 1982). It is predicted that due

to environmental homogeneity of agroecosystems (further discussed in section 1.7.1), genetic variation would be further eroded in agrestal weeds (Barrett 1988). Response to selection is largely determined by amount and pattern of genetic variation (Chapter 1, Endler 1986), and hence will determine the potential of species to invade and successfully colonize new habitats.

The broad tolerance of weed species to environmental conditions has often been acknowledged as an important component of their invasive power. Baker (1965) introduced the phrase "general purpose genotype" to describe the adaptive phenotypic plasticity inherent in weedy plant species, i.e., variation in phenotypic expression of the genotype which enhances survival and reproduction under diverse environmental conditions. In fluctuating environments where temporal and spatial variation may be too unpredictable for genetic variability to track, phenotypic plasticity would allow survival and reproduction over a range of conditions (Schlichting 1986; Sultan 1987). Plasticity is predicted to be crucial in agroecosystems where variation in disruptive and directional selection within and between seasons (and plant lifetimes) is the rule (Bradshaw 1965). To accomodate such fluctuations in environmental parameters, an inverse relationship between genetic variability and plasticity had been predicted (Bradshaw 1965; Sultan 1987). To date there has been no

consensus as to the presence or direction of such a tradeoff (Schlichting 1986), with negative (Marshall and Jain 1968), positive (Wilken 1977) and no (Schlichting and Levin 1984; Scheiner and Goodnight 1986) relationship detected.

The presence of high levels of plasticity in a given trait may buffer the mean expression of that trait from directional selection, slowing population differentiation (Bradshaw 1965; Sultan 1987). However, Schlichting (1986), and MacDonald and Chinnappa (1989) found expression of trait plasticity (amount and direction) was independent of the mean of the trait, and population divergence in both aspects is possible.

A proper assessment of colonizing ability of weedy species must estimate both genetic variability and phenotypic plasticity in life history traits among and within populations. The potential for successful growth and reproduction, and subsequent adaptation to the agricultural habitat will depend on the expression of phenotypes tolerant of biotic and abiotic conditions within novel environments.

1.2 Thesis organization

In this thesis I investigated the colonizing biology of Solanum ptycanthum Dun. (eastern black nightshade) in Ontario. This weed is a recent colonist of agrestal habitats, but has been a member of the ruderal flora for approximately 150 years. In Chapter 1, I give a brief

summary of the taxonomy and biology of Solanum ptycanthum, outline its historical migration pattern and northern range expansion, and the role that the importation of tomato transplants from the S.E. United States may play in the establishment of northern weed populations. This chapter ends with the development of hypotheses, and rationale for the experimental design employed. Chapter 2 assesses variability in the breeding system of EBN between possible source and colonial populations by estimating levels of outcrossing in agrestal habitats. Chapters 3,4 and 5 contrast the genetic and plastic variability of ruderal and agrestal populations under conditions associated with agroecosystems. Chapters 2 through 5 are presented as independent papers, with separate introductions, methods, results and discussions, incorporating the findings of prior chapters. Chapter 6 summarizes the results of each of these individual studies, and discusses their implications.

1.3 Study organism

1.3.1 Taxonomy and distribution

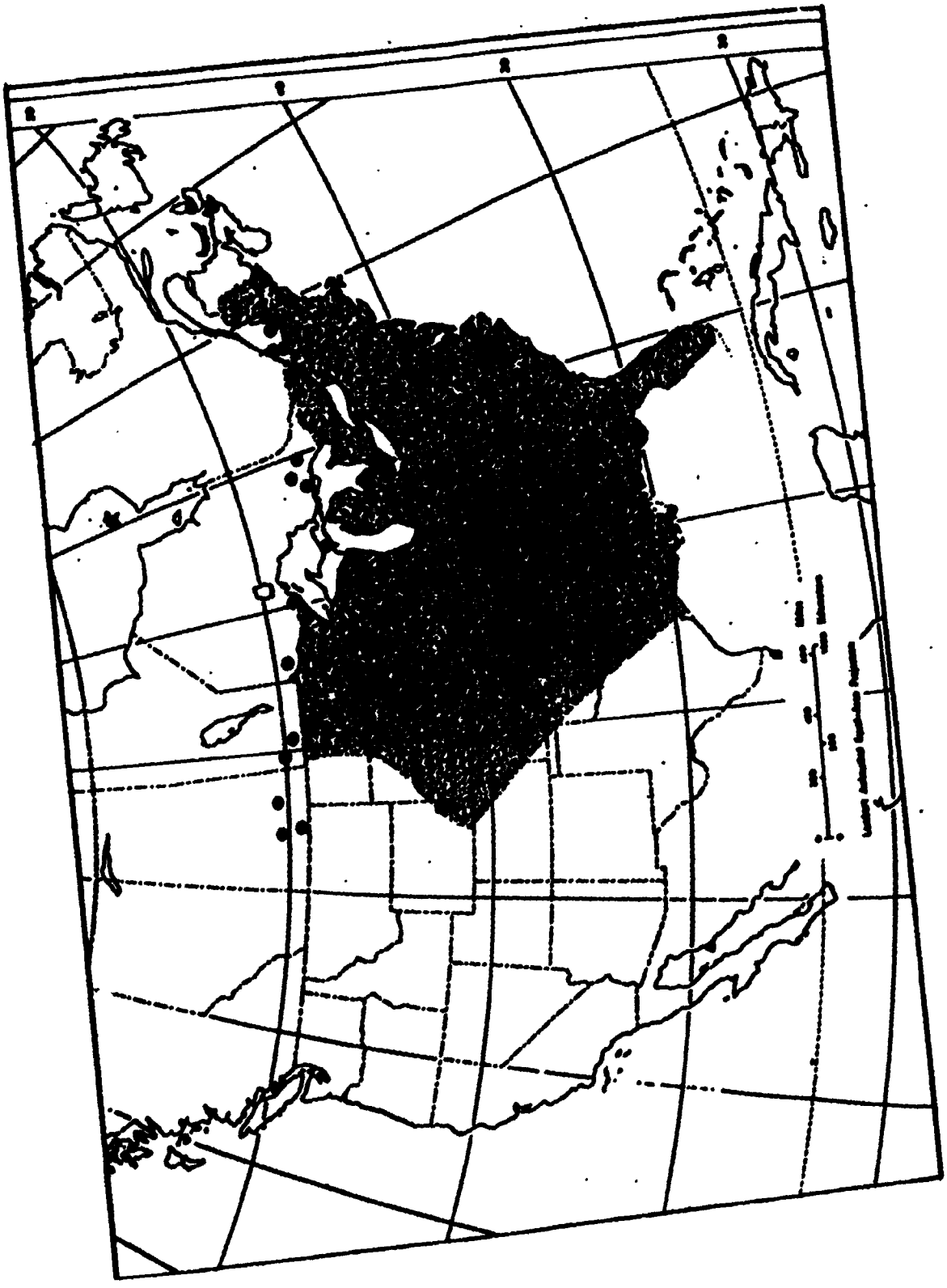
Solanum ptycanthum Dun. (eastern black nightshade) is a member of the taxonomically difficult section Solanum (also referred to as Morella or Maurella; Edmonds 1979). This section contains the Eurasian species S. nigrum L., and is also known as the "Solanum nigrum complex", the "black nightshades". World-wide, the section constitutes a group

of 30 annual or short-lived perennial, herbaceous weedy species (Schilling 1981). Of the 11 species found in North America, four (S. americanum Mill., S. nigrum L., S. ptycanthum, and S. sarrachoides Sendt.) are economically important weeds (Ogg, Rogers and Schilling 1981; Ogg and Rogers 1989). All but S. nigrum (2n=6x=72) are diploid (2n=2x=24) (Edmonds 1979). Both S. americanum (American black nightshade) and S. ptycanthum are native to eastern North America, while S. sarrachoides (hairy nightshade) is of South American origin (Schilling 1981).

In Canada, S. ptycanthum and S. sarrachoides are the most agriculturally important weed pests (Bassett and Munro 1985). S. nigrum is rare, although locally abundant in Canada except in a few disturbed areas of southern British Columbia, and can be easily distinguished from the proceeding two species by a number of characters (Bassett and Munro 1985). No confirmed specimen of this species was observed in Ontario during the duration of this research. In the United States, S. americanum is primarily distributed in the more frost-free areas of southern Georgia and Florida, west to California (Schilling 1981; Ogg and Rogers 1989). According to Bassett and Munro (1985) S. americanum is "very rare in Canada", but since 1986 I have found three separate localities in southwestern Ontario. One of these populations has successfully reproduced for 3 consecutive years (1987-1989; accession verified by E.E. Schilling).

Solanum ptycanthum is the most common nightshade species east of the Rocky Mountains, extending from the Gulf of Mexico northward into Ontario, with sporadic records from Quebec, Manitoba, Saskatechwan and the Maritimes (Fig. 1.1; Ogg and Rogers 1989; Bassett and Munro 1985). Historically, its pattern of northward migration parallels those of a number of other broad-leaved weeds now common in Ontario (Weaver 1985; Warwick 1990b). This is further explored in section 1.4. S. ptycanthum is a serious weed of a number of crops (e.g., oats, peppers, peas), but causes the most damage in tomato and soybeans (Weaver, Smits and Tan 1987; Ogg and Rogers 1989). Much information is known about the biology of S. ptycanthum as an agrestal, but the dynamics of ruderal populations are little known. For example, in a recent review of the nightshades, ruderal habitats are completely ignored (Ogg and Rogers 1989). These habitats harbour populations that have evolved under very different selection pressures (Barrett 1988; Blais and Lechowicz 1989), and represent an important component of the species genetic structure. Ruderal populations of S. ptycanthum have also naturalized in non-anthropogenic, highly disturbed habitats such as beaches and river banks. Colonization of such non-anthropogenic habitats by agrestal weeds is relatively rare, but a few examples are well known (e.g., Xanthium strumarium, cocklebur; Weaver and Lechowicz 1982).

Fig. 1.1 Present distribution of Solanum ptycanthum in North America. Dots indicate individual herbarium records. Adapted from Basset and Munro 1985, Schilling 1978, and Ogg and Rogers 1989.



1.3.2 Species description

S. ptycanthum (hereafter referred to as "EBN") is a summer annual or short-lived perennial (Schilling 1981). In agrestal habitats, and northern locations, it has a strictly annual life cycle, and reproduction is solely by seed. The breeding system is described as facultatively autogamous (Schilling 1978) but actual rates of outcrossing have not been previously reported. Breeding system is an important determinant of amount and pattern of genetic variation within species (Hamrick and Godt 1990) and will be addressed in Chapter 2. Bumblebees (*Bombus* spp.) are the most important pollinators of nightshade species (Whalen 1978), but EBN is also visited by a variety of syrphid flies and other insects.

Floral initiation is determined by growth stage rather than photoperiod, and flowering commences after the formation of the fourth to sixth leaf primordia in northern populations (Quakenbush and Andersen 1984; Weaver unpubl. data), and in southern populations, after the eighth to tenth (see Chapter 5). The growth habit is sympodial and indeterminate, and an umbellate inflorescence of up to six flowers is produced in the axis of each branch (Bassett and Munro 1985). Once flowering has been initiated, both vegetative and reproductive tissues are produced until frost kills the plant (Ogg and Rogers 1989). The fruits are berries which contain 50-110 seeds each (Ogg *et al.* 1981;

Chapter 2). Ripening fruit turn from green to black, and seeds are viable beginning at the green, translucent stage (Quakenbush and Andersen 1984). The berries are attractive to a variety of animals (e.g., birds, rodents and foxes), which then act as dispersal agents (Bassett and Munro 1985). In agroecosystems, the major route of dispersal of many weed seeds is via agricultural machinery (McCanny and Cavers 1988). EBN berries stick to machinery during crop harvesting, and are distributed both within and among fields (Ogg and Rogers 1989). Not only do EBN plants reduce crop yields through competitive interference (Weaver et al. 1987), but the presence of the berries can also reduce the value of a crop to zero by contaminating the seeds (e.g., soybeans and peas), causing mould, and preventing harvesting (Ogg and Rogers 1989).

All stages of growth are highly variable, which is one of the reasons this species presents taxonomic difficulties (gross phenotypic similarity between species, and "genetic variation" are the other reasons cited; Edmonds 1979). As is common with most weedy species, many studies (reviewed in Ogg and Rogers 1989) have reported extreme morphological flexibility, but the genetic component of this variability has not been distinguished from environmentally induced variability. The amount of time required to reach the reproductive state varies from four to eight weeks and is dependent on crop planting time, competition, temperature,

soil nutrients and moisture, and herbicide application. Seed maturation time is also determined environmentally. Within two to six weeks of flowering, viable seeds are produced (Quakenbush and Andersen 1984; Thomson and Witt 1987). Reproductive output is contingent on both abiotic and biotic factors, and ranges from a few berries under stress conditions to thousands of berries per plant with unrestricted growth (Bassett and Munro 1985; Ogg and Rogers 1989). Genetic variability in these life history traits, both within and among populations has been implied (Ogg et al. 1981), but not documented.

Schilling and Heiser (1979) found that pollen fertility of F_1 hybrids among different populations of S. ptycanth originating from eastern United States, varied from fully fertile to almost sterile, suggesting intraspecific differentiation. They also noted that morphological differentiation had not accompanied crossing heterogeneity in those populations studied. No Ontario populations were included in this study.

In the spring, seeds begin to germinate once maximum daily air temperatures approach 20°C (Ogg and Rogers 1989). Depending on latitude, seedling emergence begins anywhere from March to May, and peaks (i.e. >80%) between April and June (Ogg and Dawson 1984; Quakenbush and Andersen 1984; Weaver unpubl. data). The flush of emergence after soil disturbance is attributed to exposure to light (Ogg and

Dawson 1984), as EBN seeds are light sensitive but to various degrees dependent upon temperature (Thomson and Witt 1987; Hermanutz and Weaver 1991). Disturbance is also vital to move seeds towards the surface because their small size (1.5-1.8 mm; Schilling 1981) limits the depth from which they can emerge to 1-3 cm (Weaver unpubl. data). Seed size variability will be addressed in Chapter 3. Emergence continues at low levels into summer, but is dependent on soil moisture levels and disturbance (by tillage in agricultural systems; Ogg and Dawson 1984). Keeley and Thullen (1983) found that black nightshade plants which emerged later (August) grew more slowly, and produced fewer seeds than plants which had germinated during peak emergence (May-July). Unlike the seeds of other weedy nightshade species, freshly harvested seeds of S. ptycanthum do not show innate dormancy (Hermanutz and Weaver 1991). Roberts and Lockett (1978) have suggested that elevated summer temperatures may induce dormancy in black nightshade, but the critical experiments have not been attempted with EBN. Seed germination occurs over wide ranges of pH and osmotic potential (i.e., water stress gradient) (Thomson and Witt 1987). Seed longevity is unknown, but 11% of black nightshade seeds buried in a fallow field in England were viable after five years (Roberts and Lockett 1978). Again, it should be emphasized that the majority of the above information is based on agrestal populations. In addition,

variability (genetic or plastic) among and within populations has not been well characterized.

1.3.3 Nightshade as an "ideal" weed

Obviously EBN possesses many of the characteristics which circumscribe an "ideal" weed (Baker 1974): germination under a wide variety of conditions, rapid juvenile growth, precocious flowering, autogamy without specialized pollinators, continuous seed production under most environmental conditions with the potential to produce copious seed numbers under optimal conditions, and adaptations for long and short dispersal.

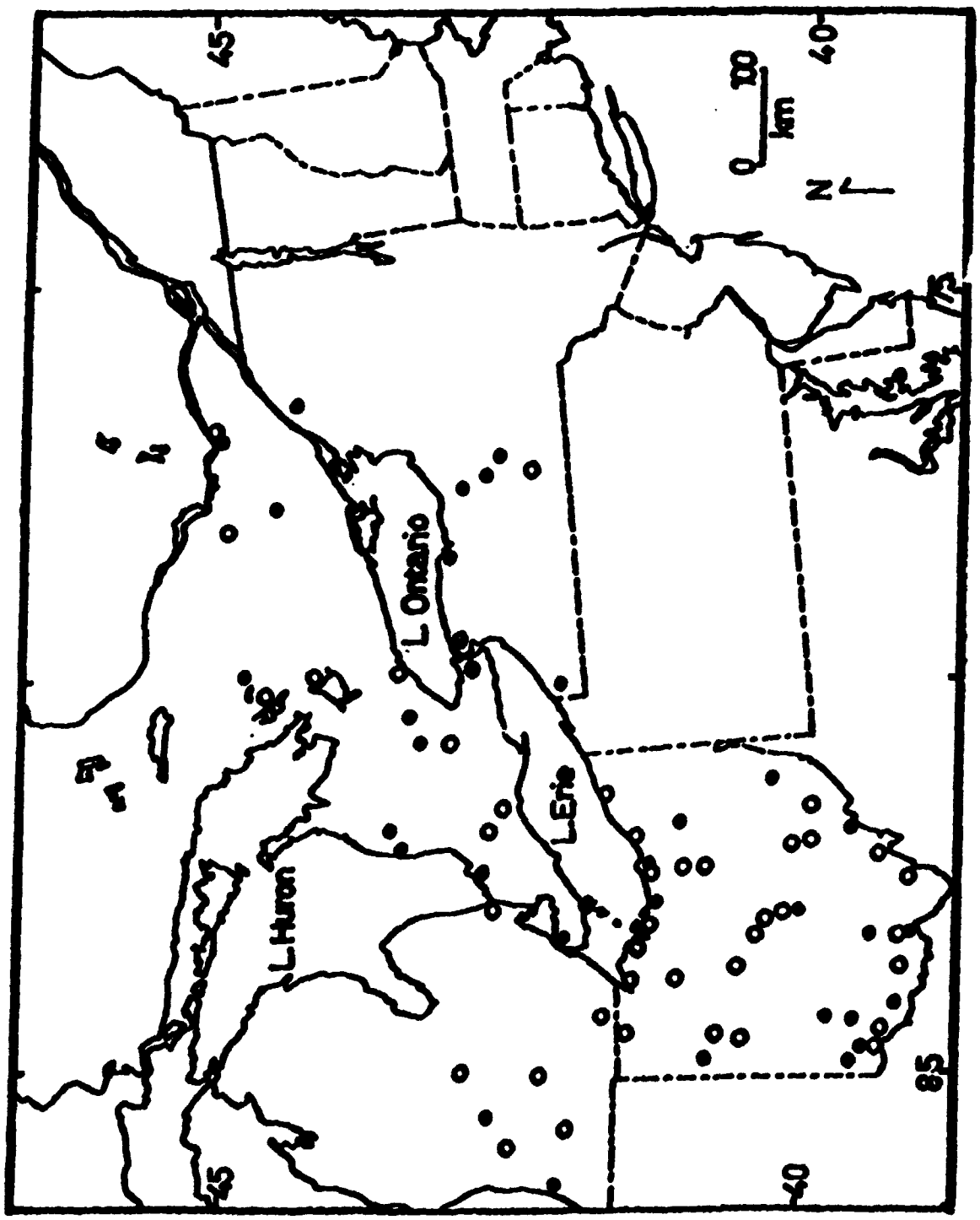
1.4 Historical background: Northern range expansion of S. ptycanthum

To understand the historical migration into Ontario, and the patterns of subsequent establishment of Solanum ptycanthum, herbarium specimens from the following herbaria were examined: National Museum of Canada (CAN), Dept. of Agriculture (DAO), University of Guelph (GUE), University of Toronto (TRT), University of Toronto Erindale (TRTE) and the University of Montreal (MTJB). In addition, agricultural records from the Weed Alert programme (1990), University of Guelph were used. Specimens from the states of Michigan (Michigan State University, MSC) and New York (Cornell University, BH), which may have acted as possible entry points into southern Ontario were also examined. Ohio (Ohio

State University, OS) specimens were considered to be important because of a similar history of tomato transplant usage (see Section 1.5.1). Although herbarium specimens are valuable in tracing such expansion, this information is compromised in a number of ways. Once a species has becomes relatively "common" or established, it is usually not consistently collected in the future. Therefore, when collating the number of counties in which EBN was present in a given time period, I have assumed it was still present in that area and was simply not collected in the subsequent time period. Extinction of these populations was unlikely, as all reports from these areas exist in a subsequent period. Collection intensity is proportional to the population density and site accessibility. Also, especially in older specimens, habitat descriptions are usually not present, and ecological information is scant.

Originally native to the eastern United States (Schilling 1978; 1981), EBN has expanded its range northward in the past 150 years. One of the earliest records in the northeastern states is 1824 in Niagara Co., NY (Fig. 1.2). Less than 25 years later (1858) EBN appeared in neighbouring Niagara Falls, Ontario, suggesting this to be the corridor of invasion into Ontario. By 1920, EBN had spread throughout Ohio and Ontario (15 counties). Its habitat was often associated with anthropogenic disturbances such as gardens, roadways and waste places. Historically this

Fig. 1.2 Distribution records of EBN in the lower Great Lakes area from the earliest records to 1919. ○:prior to 1900; ●:1900-1919. Earliest records ⓘ:in Ontario (1858) and in NY (1824).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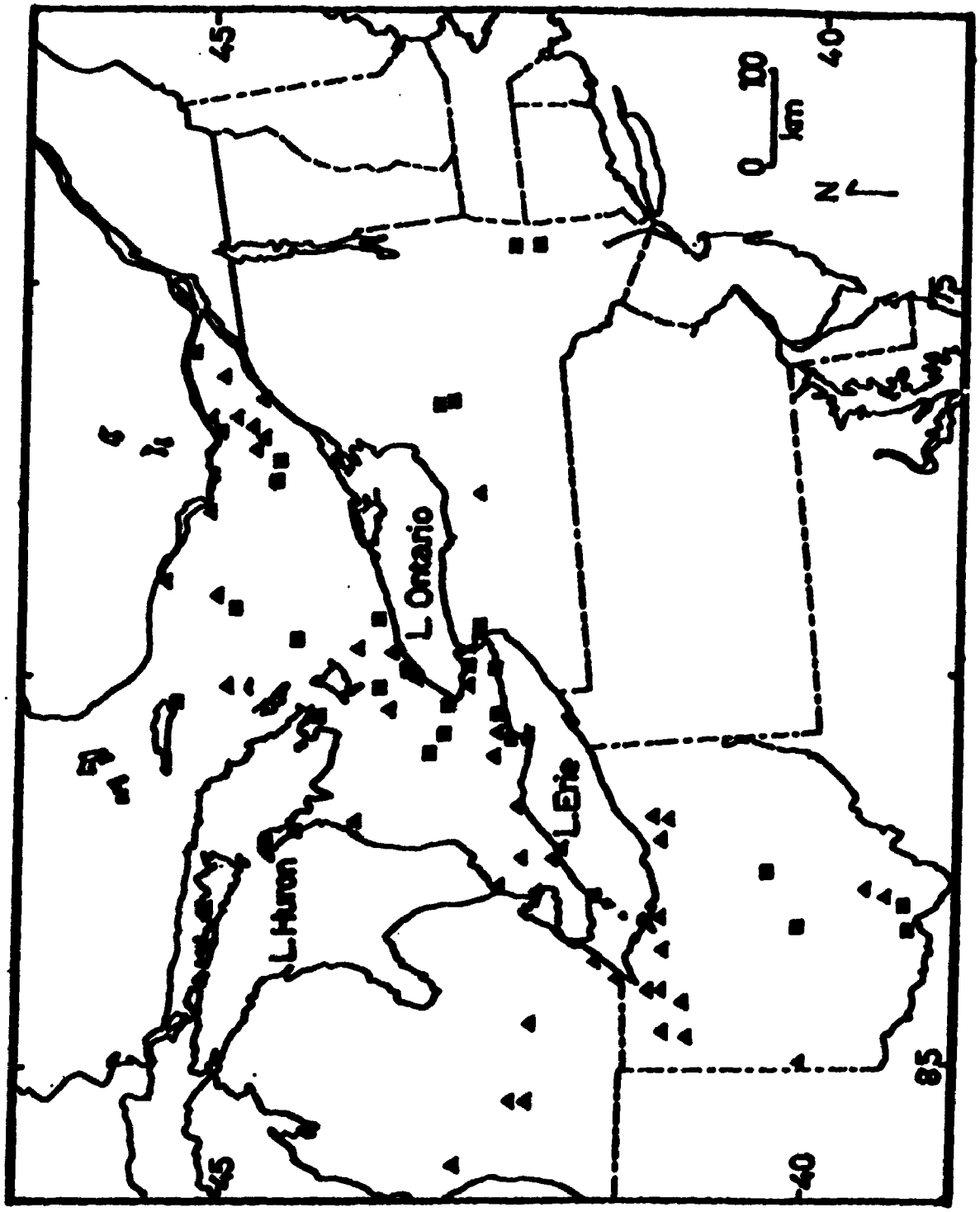
40
80
km
0
N
L Ontario
L Erie
L Huron
85
45
40

dispersal pattern follows the oft described avenue of invasion (Baker 1965; 1974; Mulligan 1965; Warwick 1990b), but unlike most other "alien" weeds (e.g., Warwick and Black 1986), EBN has also invaded and become established in "native" habitats associated with natural disturbance regimes such as beach (e.g.: Point Pelee, 1884), dune and river habitats. As well, open and shaded rich woods were successfully colonized. For the next 40 years expansion occurred into counties around major populations centres such as Ottawa and the Toronto-Hamilton corridor and into the northern districts of Ontario (Fig. 1.3). Collections during this period reflect further colonization of "native" and anthropogenically generated habitats. EBN maintained relatively low densities during this period. By 1960 EBN had been recorded in 27 counties and districts, but it was rarely recorded in agroecosystems. Only three such records existed in Ontario prior to 1960. According to examined records, EBN was not a widespread weed in either New York or Michigan during these times.

The shift into agroecosystems occurred in the early 1960's (Fig. 1.4). During this time period, records indicate EBN simultaneously invaded a variety of crops from tomatoes to cereals, throughout Ontario (Alex 1964). This expansion was also evident in the United States (Ogg and Rogers 1989). Holm *et al.* (1977) have linked this invasion to major changes in cultural methods, such as increased use

Fig. 1.3 Distribution records from 1920-1959. ■:1920-1939; ▲:1940-1959.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40
100
km
N
L Ontario
L Erie
L Huron
85
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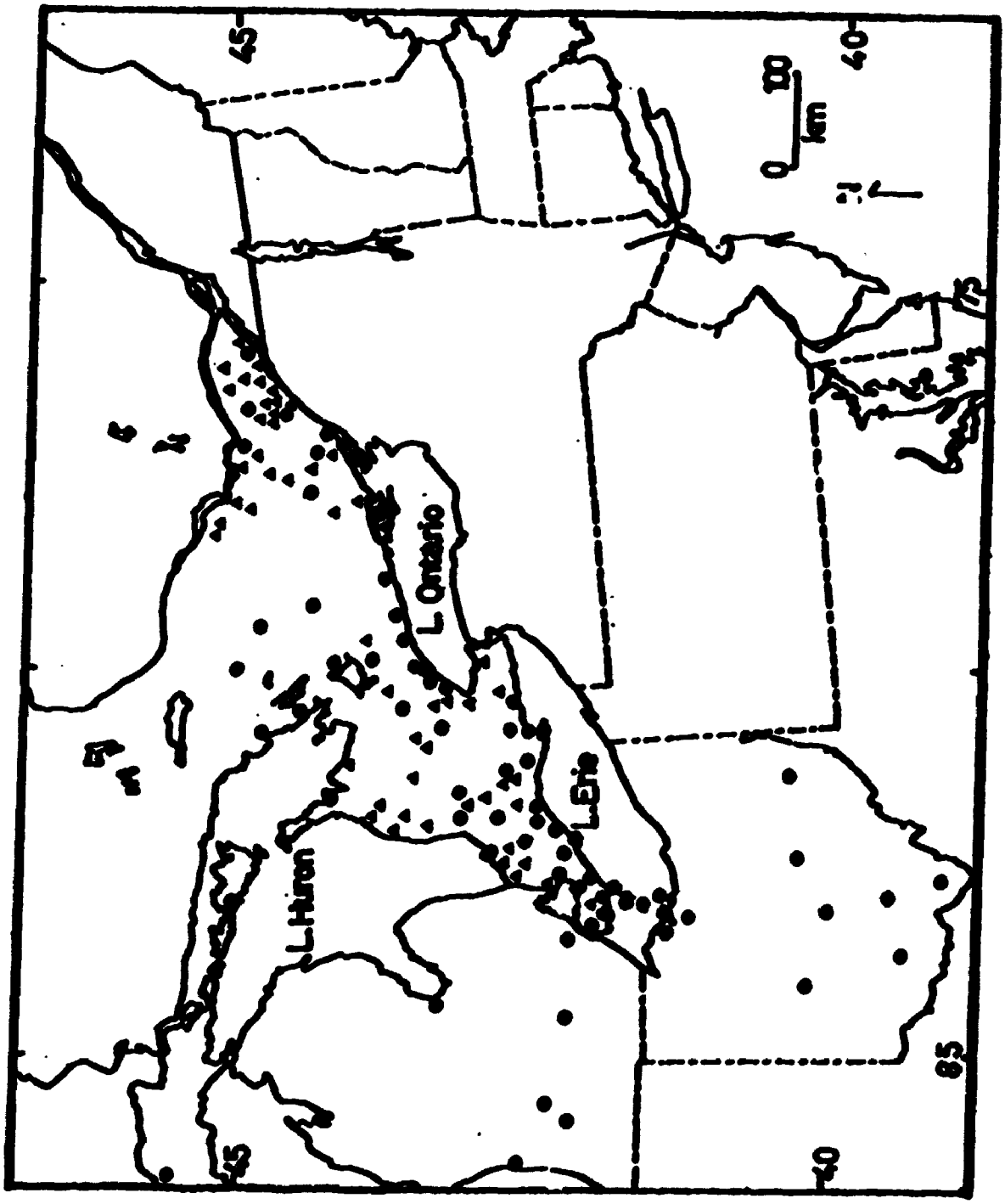
40

Fig. 1.4 Distribution records from 1960 to the present. Included are agricultural records from the Weed Alert programme. ●:1960-present; ▲:Weed alert records 1980-1990.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80

of dinitroaniline herbicides (e.g., trifluralin), with concomitant decreases in cultivation and crop rotation. These herbicides, to which nightshade species are relatively more tolerant (Ogg and Rogers 1989), significantly reduced populations of other, more competitive weeds, allowing subsequent nightshade invasion. However, it did not actually become an important pest in Ontario (i.e., high densities) until after 1980. The 1980 Weed Survey (Ontario Ministry of Agriculture and Food) did not include EBN in the list of serious weeds of agroecosystems. Since then EBN has become one of the most economically important weed species in Ontario (A. Hamill, pers. comm.), and has invaded the majority of agriculturally active counties (Fig. 1.4). Such rapid expansion may reflect the large increase in soybean hectarage during the 1970s, as it has in other broad-leaved weeds with a similar migration history (Weaver 1985). Nevertheless, in Ontario tomato hectarage has remained fairly constant since the 1960s. In 1972, soybeans covered 162,000 ha while tomatoes covered only 9200 ha. By 1984, the soybean area had jumped to 412,000 ha compared to 12,400 ha in tomato production (Weaver 1985; R. Garton pers. comm.). With the advent of machine harvesting of tomatoes, and custom combining of soybeans in the early 1970s, gene flow between areas would have been significantly increased, as seeds were spread among fields via machinery (McCanny and Cavers 1988). Movement by machinery is especially efficient

for nightshade dispersal as the berries readily adhere to the equipment and the small seeds are easily distributed (Bassett and Munro 1985). Within fields (populations), this mechanized dispersal acts to increase the small genetic neighbour sizes generated by high levels of selfing and low levels of fruit dispersal (Levin 1988; Hamrick and Godt 1990) away from the parent plant (pers. obser.). The action of the machinery breaks down the berry structure and broadcasts the seeds, thus contributing to the spread of genotypes throughout each field.

Northern range expansion has not been limited by photoperiod or delayed planting (as with Xanthium strumarium), or by a lack of seed production due to large, slow ripening fruits (as with Datura stramonium) (Weaver 1985). To date, adequate control of nightshade has not been obtained, especially in solanaceous crops (tomato, pepper, potato, tobacco etc.) (Weaver et al. 1987).

In summary, colonization of agroecosystems across Ontario was catalysed by: a) changes in cultural practices, fostering initial invasion; b) large scale expansion of appropriate crop lands and c) mechanized dissemination.

1.5 The role of imported transplants in range expansion

1.5.1 Introduction

Trading in agricultural commodities, be it seed or plants, has led to many accidental pest introductions (Baker

1986; Crawley 1987). In Canada, many of our economically important agricultural weeds were introduced in contaminated seed lots (Alex 1982). Invasion of climatically similar habitats has occurred many times in the past, as evidenced by the well documented colonization of "mediterranean-type" ecosystems in California, Chile and Australia by pests (both plant and animal) originating from Mediterranean countries of Europe and Africa (Groves 1986). Introductions of "alien" species have resulted in the transformation of entire communities (Mack 1981), while other species have had major economic impacts (Berlocher 1984).

Tomato growers in Ontario, Ohio and Michigan have imported tomato transplants from Tift Co., Georgia (GA) since the mid-1940s, and from Merryhill, North Carolina (NC) since the early 1980s. Although EBN had existed in ruderal habitats in Ontario prior to 1900, and it is generally assumed these ruderals spawned the radiation into agroecosystems (Hamill, pers. comm.), weed contamination of these transplants in the past may have contributed to the establishment and genetic constitution of Ontario infestations. Rarely does such a defined point source of entry exist, one which can be readily inspected and contaminants enumerated, and then compared to resident genotypes (subsequent chapters).

The majority of transplants (>95%) originate from GA, with only the late maturing varieties imported from NC at

season's end in June. On these southern farms, transplants along with inclusive weeds, are harvested intact as a mat of roots and adhering soil, packed into crates and shipped northward to distribution centres. All these transplants are grown by a small number of operators (<10) within a very small area around Tifton in southern GA, and by one operator (R.J. Reynolds Co.) in NC. The number of plants imported is staggering: in 1988 400,000 crates were shipped out of Tift Co., GA. With roughly 1000 transplants per crate, contamination by seeds in adherent soil and accompanying weed seedlings represents a plausible colonization route from the south. Although both the Georgia and North Carolina Departments of Agriculture certify transplants as "pest free", many Ontario tomato growers believe the present nightshade infestation is a result of transplant contamination. This would certainly be an unique method of direct colonization from the original area of radiation. In order to assess possible gene flow from the south, via seeds and/or juvenile plants, crates of transplants were inspected before they were distributed to the growers in 1987-89.

1.5.2 Methods: transplant screening and sample processing

Transplant inspection was done at the Sun Parlour Growers Cooperative in Leamington, Ontario over a 3-4 week period commencing in mid-May, 1987-89. The transplants are distributed by the H.J. Heinz Co., to their contracted growers in Essex, Kent and Lambton Co., which comprises the

majority of tomato growers in southwestern Ontario. The other major tomato growing region in Ontario is Prince Edward Co. (Fig. A.1), where Heinz also distributes southern transplants.

Each transplant crate inspected was screened for the presence of weedy plant species, and the adherent soil sampled roughly in proportion to amount present. Each crate contains 3-5 kg of sandy soil, of which 0.5 to 1.5 kg was subsampled. Crates were sampled at random from the selection of transplant farms represented on each sampling date. All species of weeds were counted and identified, and any nightshade plants encountered were transplanted into the greenhouse at the Harrow Research Station for electrophoretic screening (Appendix A). The soil was kept in plastic bags at 4°C until processed. The soil was weighed (moisture was less than 3%), and gently washed through a set of soil sieves (upper No.7 Canadian Standard with mesh size of 2.8 mm, lower No. 35 Canadian Standard with mesh size of 0.5mm). Small seeds such as pigweeds (Amaranthus spp.) and lambsquarters (Chenopodium spp.) are retained with the latter sieve size (Benoit 1986). In an effort to further separate organic and inorganic fractions, the 1987-88 samples were rewashed using Malone's (1967) method. Since the efficiency of seed detection did not increase using this technique, the 1989 samples were simply washed through the sieves. To gauge the efficiency of seed

recovery after washing, two samples (1988) were spiked with known numbers of nightshade seeds: 88 and 89% of these seeds were recovered upon sorting. Each washed sample was sorted and seeds identified. For each species, the number of seeds per sample was standardized by weight (number per kilogram), and the average number of seeds/kg for each source locality was calculated including only those samples with seeds present. Results concerning weed species other than EBN are presented in Appendix B.

1.5.3 Results and discussion

In 1987, no nightshade contamination via seed or plant was found in any of the 41 crates sampled (Table 1.1). The number of crates which can realistically be sampled from either source, is very low in proportion to the number imported (<0.1%), so that the absence of nightshade may simply reflect this small sample size. In 1988 the number of crates sampled was more than tripled to 141. No nightshade seed or plants were found in GA crates, but 75% of the NC crates had on average 13.5 plants/crate (Table 1.1). These plants ranged from seedlings less than 5 cm in height to flowering individuals in excess of 15 cm. The two NC crates harbouring substantial seed burdens of EBN were also laden with juvenile plants (>50/crate).

In 1989 nightshade contamination of NC transplants was extremely high. Over 50% of examined crates contained EBN plants, and in much greater abundance than in 1988

Table 1.1 EBN contamination of tomato transplants imported from Georgia and North Carolina. Burden evaluated by seed and plant density, and the proportion of crates contaminated (%) for each measure from 1987-89. Seed burden evaluated by mean number of seeds/kg, and plant burden by mean number plants/crate. (S.E. in parentheses). Each crate contains 3-5 kg of soil

			Seeds		Plants	
Year	Source	crates (#)	%	Mean # /kg (S.E.)	%	Mean # /crate (S.E.)
1987	GA	36	0	0	0	0
	NC	5	0	0	0	0
1988	GA	133	0	0	0	0
	NC	8	25.0	25.3 (7.35)	75.0	13.5 (7.16)
1989	GA	79	1.3	1.9	0	0
	NC	16	31.3	11.7 (5.73)	56.3	67.8 (12.07)

(Table 1.1). The plants ranged in size from 5 cm to 25 cm (these larger plants had been "topped", i.e. the tops of the transplants are cut before packing, to fit into the crates). Most of these larger plants had begun to flower. In contrast, the seed burden of NC crates was much lower than plant contamination. Perhaps the seeds had germinated just before harvest, as many seed coats were found in the crates with high numbers of nightshade plants. Sample dates (i.e., shipments) varied considerably in their contamination level. For example, all the nightshade seeds and 8 of the 9 crates with plants found in the 1989 NC transplants, originated from a single shipment (load 122).

One of the 79 GA crates screened actually did contain EBN seeds (Table 1.1), but the contamination level was low. These seeds appeared to be infected with fungus (tentatively Alternaria sp.; Traquair pers. comm.) and were not viable. Preliminary data suggest that seed mortality due to fungal infection may be an important factor regulating seed bank dynamics (Hermanutz and Traquair unpubl. data).

Given the number of crates imported into Ontario, detected levels of weed contamination have the potential to act as primary infection "foci" in the colonization of localities that employ transplants. The large number of foci, their repeated nature and disjunct distribution, combine to accelerate the rate of spread (Mack 1986). As well, the continual input of southern genotypes of EBN, as

well as other imported weed species (Appendix B), may have had major consequences for the population structure. Such inputs would dampen founder effects and subsequent genetic bottlenecks, by periodically introducing flushes of genetic variation from the south/centre of the species range, thus increasing or maintaining genetic diversity (Barrett 1988).

It is tempting to postulate that the relatively recent (i.e., 1980s) expansion of EBN in agrestal habitats may have been due to the large input of NC genotypes via these imported transplants, as it coincides with the start of importation from NC. However, as all NC plants screened were fixed for the alternate Pgm-2 allele compared to the Ontario populations (Chapter 2; Appendix A), it is unlikely that NC plants contributed to the increase in abundance.

It should be stressed that although current nightshade contamination of GA transplants is low, this may not have been the case in the past. Direct evaluation of historical burdens of EBN from GA is not possible, but present levels of contamination by other species (Appendix B) suggest the possibility of past importation of EBN. The same Pgm-2 genotype (SS) found in Ontario plants does occur in GA (Appendix A), and may have been the original colonizer of northern agrestal habitats.

The pertinent question, now that contamination has been confirmed, is whether these seeds and/or plants can successfully establish in fields of transplants.

1.6 Can nightshade imported with tomato transplants successfully establish in Ontario?

1.6.1 Introduction

Groves (1986) has partitioned the "invasion" process into three phases in time. INTRODUCTION sees the seed (or plant) arrive, germinate and grow in a new region. This may occur in several different sites, at several different times. COLONIZATION is achieved if that plant reproduces itself, disperses and multiplies. NATURALIZATION is simply the successful proliferation of that species over time. Evidence presented in the previous section documents the potential for direct introductions of southern genotypes into agroecosystems in southwestern Ontario. Many factors, both physical and biological, will determine if this introduction phase will be successful and develop into an actual colonization. The majority of introductions never reach the colonization stage (Crawley 1987). Can these southern genotypes, once introduced, successfully colonize?

A genetic marker was needed to differentiate nightshade from the two sources, resident and import. Electrophoretic screening detected a single polymorphism (Appendix A). EBN individuals imported with transplants from NC are fixed for the fast allele at Pgm-2 allele, compared with the slow allele resident (Ontario) populations. This polymorphism was used to evaluate the frequency of successful nightshade colonization in tomato fields planted with NC transplants.

Although EBN has been established in Ontario agroecosystems prior to these introductions from NC, documentation of establishment and reproduction of NC imports provides an example of how GA imports may have initiated the agricultural radiation, and/or contributed to its genetic structure. Direct documentation of colonization by GA individuals is not possible due to the low burden of imports (Table 1.1), unknown genotype frequencies of the imports, and the inability to distinguish the SS genotype from resident nightshade genotypes.

1.6.2 Methods

In the present example, the probability of an EBN seed successfully reaching the reproductive stage is probably much lower than that of an intact, fully rooted juvenile plant. Environmental cues and conditions must be appropriate for germination (Chapter 3), and mortality due to pre- and post-emergence herbicides (Chapter 4) and cultivation would be great. The highly vulnerable early seedling stage is circumvented by the juvenile "transplant". The contaminant EBN plants resemble tomato transplants, and are often planted as tomatoes by field labourers. Nightshades which have been planted as tomatoes can often be distinguished from resident seedlings by their comparatively large size and by their location directly in the planted row, at the appropriate spacing. Obviously a resident seedling may still germinate and grow within the tomato row.

During the growing season weed control efforts, such as hoeing, selectively remove those individuals not in the row, further increasing mortality of the EBN seedlings which had arisen from seeds. For these reasons, importation of weeds as "transplants" may enhance the probability of establishment and future reproduction.

To ensure that all established nightshade plants present in tomato crops were fixed for the slow Pgm-2 allele, twelve additional populations were sampled in 1988 and 1989. Three Michigan populations that received GA transplants were included, as were additional samples of ruderal populations from Point Pelee National Park (Essex Co.) and Rondeau Provincial Park (Kent Co.).

In 1988 a single field which had received NC transplants was randomly sampled. In 1989, three growers who had received crates from the same highly contaminated NC transplant shipment (load 122) were selected. Each grower also had adjacent fields planted with the same tomato variety (Heinz 722) originating from GA for comparison. All tomatoes were planted on beds in double rows. Samples were harvested in August and September, just before the tomatoes were harvested, in order to select reproductively mature individuals. To distinguish those individuals that likely originated as transplant contaminants and had been planted as tomatoes, I sampled plants growing on the tomato beds, directly in the planted row if possible. Tnis sampling

regime maximizes the probability of encountering an "imported" EBN plant, rather than yielding an overall frequency of each genotype. In one field I separately sampled nightshade plants between the rows of tomatoes. These plants would most likely be resident. In most cases the plants growing within the rows were much larger and had dispersed berries long before individuals not directly within the tomato row. Nightshade densities within the tomato rows were much lower in the fields that had received GA transplants.

Leaf tissue was harvested from individual nightshade plants. Enzymes were extracted as detailed in Appendix A, and Pgm-2 genotypes scored using the cellulose acetate electrophoresis system.

1.6.3 Results and discussion

All individuals sampled from tomato fields that had received GA tomato transplants in both 1988 and 1989 were the same Pgm-2 genotype (SS), as were ruderal individuals (Table 1.2). Thus, at least in Essex and Kent Counties it can be assumed that all resident EBN are this genotype. With only a single polymorphic locus, establishment of the slow genotype from GA cannot be detected, hence successful colonization by GA imports in the past is equivocal. However, given the present low contaminant levels of the GA transplants, this is unlikely to be a current problem. It also suggests that if the FF genotype was introduced from GA

Table 1.2 Frequency of the F allele[1] in EBN populations which received tomato transplants from GA, and in two ruderal populations

Sampling date	Location	# populations	N	% FF
August 1988	Munro Co. (Michigan)	3	44	0
"	Essex Co. (Ontario)	3	35	0
Sept. 1989	Essex/Kent Co.*	2	58	0
"	Essex Co.	4	101	0
TOTAL		12	238	0

[1] No heterozygotes (Pgm-2 SF) were detected
* Ruderal populations

in the past, it did not establish successfully. A much larger Ontario survey, including different crops, would have to be undertaken to confirm this hypothesis.

Successful establishment of the Pgm-2 FF genotype from NC could not be detected in the single field sampled in 1988 (Table 1.3). During that year, the sampling procedure did not concentrate on detecting "imports", and the sample size may not have been large enough to detect a low incidence of establishment. Mortality levels between crate inspection and planting are unknown. In many cases the transplants are screened by farm labourers before planting. This mortality, in conjunction with the low burden detected (Table 1.1), may have been below some "threshold" density required for successful establishment.

In 1989, southern genotypes established and reproduced successfully (Table 1.3). A single heterozygous individual was detected, and since it was located within a row, it most likely originated as an import from NC. The contamination level of the transplants in 1989 seemed to be in excess of some unknown "threshold" number needed to colonize successfully. On average, each crate in load 122 contained 76 (± 44.38) nightshade plants. In a 14 ha field, grower 2 planted 250 crates from load 122, which would have potentially introduced 1,000 (lowest #/crate) to 34,000 nightshade plants into this field! The actual frequency of establishment differed between growers, even though all

Table 1.3 Frequency of the FF genotype of EBN in populations sampled from three growers who had received tomato transplants from NC and GA in 1989, and resampled the following spring (1990). A single field receiving NC transplants of unknown origin was sampled in 1988

Date	Grower (Location)	Source	N	% FF
Aug. 1988	-	NC	20	0
Sept. 1989	1 rows[a] (Kent Co.)	NC	81	9.9
		GA	50	0
"	2 rows (Kent Co.)	NC	100	67.0
		GA	27	0
"	3 rows (Essex Co.)	NC	105[b]	80.9
	between	NC	30	6.7
		GA	54	11.1
June 1990	2	*	109	13.8
	3	*	27	70.4

[a] Plants sampled in tomato rows or between rows. See text for description of the sampling regime.

[b] One heterozygote was detected.

* Planted with crops other than tomato (see text).

received tomato transplants from the same load. Successful establishment of the FF genotype varied from 9.9% (grower 1) to over 80% (grower 3) (Table 1.3). Variable establishment success may be based on differential screening efficiency before planting, environmental variability between fields, and/or variability in contamination within the load of transplants. The rather low frequency found in the crop of grower 1 reflected the poor health and high mortality rate of the tomato transplants. Many of the nightshade plants had died or did not reproduce. The tomatoes from GA growing in adjacent fields were very healthy. Although all crates examined in this load contained nightshade plants, the actual number of plants/crate was quite variable (Table 1.1). Perhaps grower 1 received a portion of the load which was less contaminated.

On the other hand, the high levels of establishment (>70%) in the other two fields reflected very healthy tomato crops. Obviously many of the NC imports had been planted as tomatoes, matured and successfully produced berries with viable seeds. The low frequency (7%) of FF genotypes sampled from between rows (Table 1.3, grower 3) reflects successful establishment from seeds imported from NC. In two of the three fields sampled, all nightshade plants harvested from adjacent fields planted with GA transplants were the SS genotype (Table 1.3), suggesting the lack of establishment of the FF genotype from GA. The FF genotype

was detected (11%) within the GA portion of the field of grower 3. There are several possible explanations for this finding. Seed contamination from the NC portion of the field, or improper designation of transplant origin in the field, must be considered, since these FF individuals were harvested from the 7 rows directly adjacent to the NC transplants. In addition, there may have been residual contamination from NC transplants planted in past years. Unfortunately, accurate information as to the prior history of the these fields (i.e., had they planted NC transplants in the past, and possible location) was not available, because most growers do not keep accurate records of such information. Lastly, there is a small probability that GA transplants were contaminated with EBN, and this was not detected. The present study can not discriminate between these alternative explanations.

Once I had documented successful growth and reproduction of these southern genotypes, the next step in the invasion process had to be evaluated. To determine if the seeds produced by these introduced genotypes could a) survive the winter conditions, and b) tolerate the various cultural regimes to emerge the next spring, I resampled two of the three growers' fields in late June 1990. Both of these growers had rotated their fields to different crops (grower 1-peas; grower 2-soybeans), so seedlings were randomly sampled throughout the entire field.

At the time of sampling, the seedlings had only emerged recently. Fourteen and seventy percent of the seedlings sampled were the FF genotype (Table 1.3). The estimates differ as a result of different practises associated with the crops, and/or other environmental factors. Detection of the FF genotype confirms that the NC genotypes can overwinter and tolerate the various herbicides applied in planting preparation, and the other stresses associated with germination and emergence. It is unknown if the individuals which emerged successfully reproduced by harvest time. As sampling regimes differed between years, the frequencies cannot be compared directly.

These results suggest that gene flow from GA in the past could have potentially initiated Ontario agrestal populations, or at least contributed to the genetic structure of the present population, if one assumes these two imported populations (GA and NC) behave similarly. Genetic diversity would be increased by the introduction of new genotypes and/or by the availability of southern plants to act as pollen parents for outcrossing. The importation of southern EBN genotypes via transplants appears to be another important component in the invasion of agroecosytems (section 1.4). Population variability between import and resident should be evaluated to gauge the potential effect of these imports on the population biology of northern populations.

As the introduction and colonization phases have been successful, why are these FF genotypes not more widespread? Several reasons may have prevented the subsequent naturalization of southern genotypes in the past. As the lag time between introduction and spread may vary (Forcella 1985), the time since initial importation from NC may have been too short. The existence of low frequency pockets of southern genotypes is certainly possible. Extensive sampling of a large number of fields which have received NC imports in the past would be necessary to document this. Alternatively, perhaps naturalization cannot occur because of the interaction of a multitude of biological and physical factors acting on the early seedling phase. Greater susceptibility of the southern genotypes to post-emergence herbicides (Chapter 4), competition and pathogens (e.g., aphids) may cause catastrophic mortality, limiting future recruitment. Combined with possible seed bank mortality (e.g., caused by fungal pathogens), slow decay without ample recruitment would lead to extinction. In addition, movement of EBN to contaminate other shorter season crops or varieties may be limited by certain life history traits, such as delayed germination and a long pre-reproductive phase. Changes in the frequency of the F allele would have to be monitored over a much longer period than has been done to document naturalization.

1.7 Experimental design

1.7.1 Population selection

The northern agrestal populations may have originated from the southern agrestal imports (as documented above), from existing ruderal populations (DeWet and Harlan 1975), or they may have been derived from northwardly migrating agrestal genotypes specialized in colonizing agricultural habitats (Baker 1974). It is certainly possible that a mixture of the above populations spawned the northern agrestal populations. The actual origins of these marginal populations cannot be reconstructed, but colonization of northern agrestal habitats may have been accomplished by both successful invasion by a subset of northern ruderal, and/or southern agrestal genotypes (genetic), or the plastic accommodation of all genotypes to novel environments (phenotypic plasticity) (Blais and Lechowicz 1989). The direct influx of nightshade plants as contaminants in tomato transplants from the south/centre of the species' distribution (GA and NC) presents an unique opportunity to study the potential pool of immigrants, and compare them to agrestal populations on the species' northern boundary (Mulligan 1965; Antonovics 1976).

The standard practise of growing plants in a "common" uniform environment to detect population divergence can obscure variability, especially if phenotypic plasticity (i.e. genotype X environment) occurs (Quinn 1987). The

degree of interpopulation resemblance can best be evaluated by examining the pattern of variation among populations in a series of environments and comparing patterns of response (Andersson 1989a). The remainder of the thesis will characterize the colonization potential of EBN by comparing variability in outcrossing rates (Chapter 2), and the relative contributions of genetic and plastic variation of presumed source and resident populations when grown under various environmental conditions. These abiotic factors were specifically selected to represent those that would be encountered upon invasion of agroecosystems in marginal northern locations: temperature (Chapter 3), and herbicide (Chapter 4) and nutrients (Chapter 5).

Populations were sampled from each of the following habitat and location "types": northern ruderal, northern agrestal and southern agrestal (Table 1.4). Southern ruderal populations could not be included in the study, owing to the rarity of such populations in Georgia (E.E. Schilling, pers. comm.). Given the contrasting latitudes and habitats of these populations, genetic differentiation in various life history traits would be expected (Blais and Lechowicz 1989). To increase the proportion of variability sampled, two populations from each type (except NC) were sampled because interpopulation differentiation can be large in autogamous species (Hamrick and Godt 1990), and it is necessary to obtain some measure of intra-type variation in

Table 1.4 Sampling locations of northern ruderal and agrestal, and southern agrestal types of EBN, specifying the habitat of origin and grid coordinates of member populations Southern Ruderal populations were not sampled due to their rarity in Georgia.

TYPE/Population	Location	Lat./Long.	Habitat
NORTHERN RUDERAL (NR)			
Pelee (P)	Pt. Pelee National Park, Essex Co., Ont.	42°03'N 82°59'W	sandy beach
Rondeau (R)	Rondeau Provincial Park, Kent Co., Ont.	42°20'N 81°59'W	"
NORTHERN AGRESTAL (NA)			
Harrow (H)	Harrow Research Station, Essex Co., Ont.	42°30'N 82°55'W	tomato field
Wright (W)	Wright's Farm, Essex Co., Ont.	42°03'N 82°55'W	"
SOUTHERN AGRESTAL (SA)			
A.			
Georgia 1 (G1)	Transplant Farm 1, Tift, Co., GA.	31°37'N 84°10'W	tomato field
Georgia 2 (G2)	Transplant Farm 2, Tift Co.,	" "	"
B.			
North Carolina (NC)	Seedlings imported with tomato transplants	35°36'N 77°23'W	*

* EBN seedlings retrieved from crates of tomato transplants. See section 1.5 for explanation.

order to assess inter-type patterns. Only one "population" from NC could be included because all imported transplants are grown in a single field at Merryhill, NC. The NC population was incorporated into the study only after the initial design had been chosen, because very high levels of import contamination were discovered subsequent to initiation of the study.

In agroecosystems a single continuous field has been used to define a "population" in this study. Ruderal populations were chosen from "naturally" disturbed areas (e.g., beaches) rather than from anthropogenically generated waste places because these habitats were perceived to be most extreme compared to agrestal populations. Also, gene flow, both historical and current, between ruderal and agrestal populations would be minimal. The two populations representing each type were similar in size and density of flowering individuals, colonization history, and environmental background, but the northern agrestal populations were much larger (>1000 plants) than the other types. Differences among types will be further described in the following chapters.

Several testable hypotheses can now be formulated. Due to a limited number of founding individuals, and genetic drift resulting from highly variable abundance levels, geographically peripheral populations tend to be genetically less variable than central populations (Brussard 1984). The

first hypothesis predicts that southern (agrestal) populations will be genetically more variable than recently established peripheral northern populations. As discussed in the introduction, Bradshaw (1965) predicted that populations with depauparate levels of genetic variability should exhibit greater levels of phenotypic plasticity in fluctuating environments. The level of phenotypic plasticity for a particular trait has been shown to evolve independently of the mean of that trait (Schlichting 1986; MacDonald and Chinnappa 1989). The concept of a "general purpose genotype", i.e. the ability of a limited number of genotypes (or even a single genotype) to tolerate a wide range of ecological conditions, suggests that a high degree of plasticity would be adaptive in colonizing species (Baker 1974). As a corollary of the first hypothesis, are peripheral populations also more plastic than central populations?

The predictability associated with plowing, crop planting and harvesting, fertilizer inputs and other environmental characteristics has led to the suggestion that agroecosystems are environmentally more homogeneous than ruderal habitats (Table 1.5; Barrett 1988; Warwick 1990a). The second hypothesis predicts that agrestal populations may be genetically less variable than ruderal populations. There is limited evidence that suggests that amount and pattern of variation change under cultivation (Barrett

Table 1.5 Ecological differences between ruderal and agrestal habitats (after Barrett 1988)

Feature	Ruderal	Agrestal
Disturbance	Variable intensity, often unpredictable	Frequent, predictable in space and time
Moisture supply/ soil fertility	Heterogeneous, resulting in a wide range of plant performance	Homogeneous, resulting from regular plowing, and inputs of fertilizer
Plant cover	Open areas and patchy distribution of plants owing to environmental heterogeneity and asynchronous seasonal phenologies	Synchronized phenology with rapid closure of stand
Biotic complexity	Variable, dependent on pattern of disturbance and successional status	Monoculture of genetically uniform crop variety, with restricted number of pests, weeds and diseases

1988). Again, this hypothesis can be extended to suggest that agrestal populations should be more plastic (Bradshaw 1965; Schlichting 1986).

Documentation of the levels of genetic variation both within and among populations (Venable and Burquez 1989), and the amount that is attributable to phenotypic plasticity is essential to understanding the potential for evolution to proceed via natural selection (Antonovics et al. 1988).

1.7.2 Generation of S_1 families

In August and September 1987, ripe berries were harvested from 5 (or in one population G1, 3) randomly selected maternal parents from populations of each type (see above; Table 1.4). Progeny derived from each maternal plant was considered to be a separate family. Berries from only three plants were collected from the G1 population because these were the only plants in fruit at the time of harvest.

Berries were stored at 4°C and 45% RH until January, 1988 when seeds were extracted by washing berries through a nytex screen. Seeds were dried overnight at room temperature. Five plants in each family were reared for one generation in the greenhouse to multiply the seed, and to minimize the variation induced by differences in environments experienced by the original maternal plants. This intermediate step is crucial given the very different environmental backgrounds of the maternal plants, but does not entirely eliminate the maternal/grandmaternal effects

confounded within genetic effects (i.e., among family or population components) (Shaw 1986). Upon flowering, plants were allowed to self-pollinate. The lack of fruit set by emasculated flowers observed in a companion study (see Appendix A) confirmed that pollen was not transferred between plants in the greenhouse. The seeds collected from each family represent a set of self-sibs. Of the five individuals grown per family, berries from one individual, randomly selected to represent the original grandmaternal lines, were harvested in April 1988, and stored at 4°C and 45% RH until August 1988 when seeds were extracted as above. These S_1 seeds were then used in the experiments detailed in the following chapters.

In the case of the NC population, contaminant seedlings (<10 cm) were randomly sampled from imported tomato transplant crates in June 1988 (see section 1.5). The seedlings were grown in the greenhouse, and selfed berries harvested when ripe. Five families were randomly selected. Seeds were handled as described above. As the parentage of of the original NC seedlings was unknown, and the NC EBN transplants were raised under different conditions later in the season, which would generate significantly different environmental maternal effects, the NC population was not formally included in the analyses in following chapters. For example, Wulff (1988) has found that individuals of Amaranthus dubius raised under warmer temperatures showed

significantly different maternal effects in germination characters.

It is well known that any genetic variability characterized under artificial conditions (e.g., greenhouse) may be totally overwhelmed by phenotypic responses in the field (Venable 1984; Endler 1986); that is, genotypic-specific responses to environmental factors are distorted. I chose to conduct the following experiments under the "artificial" conditions of the greenhouse and/or germination incubators because I was interested in the potential of these populations to invade novel habitats (Vickery 1974), and hence I subjected populations to "extreme" levels of environmental factors investigated. All environments selected reflect levels which may be encountered in either ruderal (e.g. low fertility, lack of herbicide exposure) or agrestal (e.g. high fertility, high herbicide exposure) habitats. Definition of the tolerance of a genotype (or family) across such a gradient allows comparison of the "potential" niche of families and populations. Bradshaw (1984) has suggested that reaction to extreme or stressful conditions best defines this potential. Also, certain environmental factors can successfully be manipulated in the field (e.g., density) but others such as nutrients and temperature cannot be precisely controlled across a wide gradient.

Given the constraints under which the the following

experiments were run (i.e., limited greenhouse and incubator space), there was a tradeoff between the number of families and populations that could be grown, and the number of levels of an environment that could be tested. The sample sizes chosen (i.e., 5 families/population; 2 populations/type) balanced the additional levels of each environment used in the greenhouse experiments, and allowed me to partition inter- and intra-population variability of each character.

CHAPTER 2-ESTIMATION OF OUTCROSSING RATES

2.1 Introduction

Autogamy is a predominant feature of annual, weedy colonists (see reviews by Baker 1974; Jain 1976; Brown and Marshall 1981; Price and Jain 1981; Barrett 1982; Barrett and Richardson 1986; Brown and Burdon 1987; Barrett 1988; Warwick 1990a). The importance of uniparental reproduction has been attributed to reproductive assurance upon colonization (Baker 1955; 1974), and to the ability of these species to rapidly saturate the new habitat with pre-adapted genotypes (Stebbins 1957). Self-pollinating species are also predicted to retain low levels of genetic diversity within populations (Jain 1976). In a recent survey of plant mating systems undertaken by Schemske and Lande (1985), weedy species which colonize highly disturbed habitats constituted the majority of the "primarily selfing" (i.e., <20% outcrossing) category. If this category is further restricted to mirror a more realistic definition of selfing (<10% outcrossing; Brown 1990), these colonizing weeds would constitute the entire "selfing" group. In their survey no distinction was made between ruderal and agrestal populations of weedy species. Without exception, the selfing species surveyed originated from diverse ruderal habitats. Agrestals were not included in Schemske and Lande's compilation, because none such studies exist. Mating system evolution in agrestals may reflect the very

different selection pressures generated by agricultural practices, as compared to ruderal habitats (Warwick 1990a).

Barrett (1982; 1988) has suggested that due to environmental homogeneity of agricultural systems, agrestals may have lower levels of genetic variation when compared to ruderals. Although few studies have explicitly compared ruderal and agrestal populations (Price and Kahler 1983; see Barrett 1988), isozyme surveys of autogamous, agrestal populations of the following annual weeds found an extremely restricted number of genotypes throughout the sampled ranges: witchweed (Striga asiatica (L.) Kuntze; Werth, Riopel, and Gillespie 1984), proso millet (Panicum miliaceum L.; Warwick 1987), velvetleaf (Abutilon theophrasti Medic.; Warwick and Black 1986), jimsonweed (Datura stramonium L.; Warwick 1990b), and barnyard grass (Echinochloa oryzoides; Barrett and Richardson 1986). Most populations surveyed were monomorphic. Low levels of observed allozyme variation within populations may result from decreased outcrossing in agrestal populations. Lack of polymorphic loci in these agrestal populations makes estimation of outcrossing rates difficult (Baker 1974; Warwick 1990b), although morphological markers such as pigmentation have also been used (Andersen 1988). To date no study has compared the outcrossing levels of annual, agrestal populations with their ruderal counterparts.

Solanum ptycanthum (Dun.)(Solanaceae), eastern black nightshade, is a weedy annual which has successfully

colonized both ruderal and agrestal habitats in eastern North America. A low pollen:ovule ratio (P/O=160) indicates its breeding system is autogamous (Schilling 1978), but as this estimate was based on only two plants, variability across its geographical range can not be assessed. S. ptycanthum is a native, diploid species (2n=24) distinct from S. nigrum sensu lato, with which it has been confused in the past (Schilling 1981). Widely distributed in eastern North America from Florida northwards (Ogg and Rogers 1989), it reaches the northern limit of its range in Ontario (Bassett and Munro 1985). It is presently expanding its distribution within Ontario. Although ruderal populations of eastern black nightshade have been established in Ontario since the mid-1800's, it has only recently (circa 1960) invaded agroecosystems (Alex 1964). During this time Ontario growers imported tomato transplants from Georgia and North Carolina which were contaminated with eastern black nightshade, thus introducing S. ptycanthum directly from the southern portion of its range, into a marginal locality (see section 1.5).

A preliminary electrophoretic survey (14 enzymes encoding 21 loci) of eleven Ontario populations (3-4 individuals/population), representing both agrestal and ruderal types, detected no allozyme polymorphisms (see Appendix A), precluding mating system estimates based on enzyme markers in natural populations. An initial screening of the southern populations revealed a polymorphism at the

phosphoglucomutase-2 locus (Pgm-2). Ontario populations were fixed for the slow allele (S), while the plants from a single North Carolina field were homozygous for a faster migrating allele (F). Both homozygotes (FF and SS) were found in a sample of eight plants from Georgia but no heterozygotes were detected.

The presence of this polymorphism provided an opportunity to test for mating system differences between agrestal and ruderal genotypes of S. ptycanthum. Synthetic field populations which differed for allozymes at the Pgm-2 locus were established within agrestal habitats in southern Ontario, to compare the potential for outcrossing rate of recently colonized northern agrestal individuals to northern ruderal, and southern agrestal individuals of EBN, which may have founded these northern agrestal populations. Floral and phenological characters which were considered to be important in determining the potential for outcrossing, were also measured for each population (Rick, Fobes, and Holle 1977; Schoen 1982).

2.2 Materials and methods

2.2.1 Population sampling and experimental design

The northern agrestal populations (W and H) were sampled from tomato fields in Essex Co., Ontario. The northern ruderal populations were sampled from sandy beach habitats in Rondeau Provincial Park (R), Kent Co., and Point Pelee National Park (P), Essex Co., Ontario. The southern

agrestal populations were sampled from tomato farms in Tifton, Georgia (GA), and from the contaminant burden of tomato transplants imported from Merryhill, North Carolina (NC). Two (or three for GA) families (i.e., progeny from a single individual) of known Pgm-2 genotype from each population were randomly chosen from five families that had been grown in the greenhouse for one generation and allowed to self, to reduce maternal environmental effects. The plants were started in the greenhouse and transplanted onto the periphery of fields of various crops on the grounds of the Agriculture Canada Research Station, Harrow, Ontario, in mid-July 1989, once flowering had commenced. All open flowers were removed from the target plants upon transplantation.

Families were replicated by planting a target plant into two different experimental areas. Each target plant (homozygous for Pgm-2) was positioned in the centre of a square meter plot and surrounded by six plants (2 families from each of 3 populations) homozygous for the alternate allele. Target plants homozygous for the allele common in Ontario (SS) were stationed in fields where there were no indigenous nightshade plants present. To prevent contamination between arrays, each array was placed at least 100 m from the nearest neighbour and separated from it by crops. This design reflected realistic weed and pollinator densities found within agroecosystems.

Schilling and Heiser (1979) detected large

interpopulational variability in F_1 fertility of eastern black nightshade, suggesting barriers to successful crossing. To screen for possible crossing barriers between northern and southern populations, which would bias outcrossing estimates downwards, two Georgia FF genotypes were surrounded by Georgia SS genotypes and vice versa. No similar comparison could be done for northern plants since the FF northern genotype has not been encountered. To determine whether the stigma is receptive upon flower opening, selected buds were emasculated one day prior to opening and stigmas saturated with a mixture of the alternate allele pollen from different families.

2.2.2 Electrophoresis

Ripened berries from each array were harvested five times from the end of August to mid-October to detect temporal differences in outcrossing rate. The berries from each sampling date were stored separately at 4°C for two months. Five berries per target plant, from each sampling date, were washed individually, and germinated at 30/20°C (14 hr light at the higher temperature). Berries contained 80-120 seeds. Ten seedlings/berry were randomly selected, and individually ground in buffer (Tris-HCl, pH=8.0, with 5µl/ml B-mercaptoethanol) and PVP (Polyvinyl-pyrrolidone). To avoid biasing the estimate by sampling only germinating seeds, imbibed seeds were ground instead of seedlings in families that did not germinate fully (>95%). Cellulose acetate electrophoresis (Helena Laboratories, Beaumont TX)

(Easteal and Boussy 1987) was used to detect heterozygotes at Pgm-2. Samples were applied to gel plates (Titan III, Helena Labs) which had been previously soaked in electrode buffer (0.024 M Tris, 0.19 M glycine, pH=8.4). Electrophoresis was carried out at 2 mA per gel for 25 minutes at room temperature. Plates were stained for PGM using an agar overlay (Hebert and Beaton 1989). The generation of SF heterozygotes upon crossing SS and FF homozygous parents, and true breeding of progeny from self pollinations of homozygous plants established the genetic basis of the polymorphism.

After the initial screening was completed, a further three berries were sampled from harvest dates that indicated outcrossing had occurred. This was necessary to obtain a more accurate estimate of outcrossing because of the heterogeneous occurrence of outcrossing within and among berries. This sampling yielded a sample size of 27 arrays (2 of the original arrays were accidentally mowed), each with 8 berries sampled over 5 harvest dates, and 10 progeny screened/berry for a total of 2050 individuals.

Outcrossing was estimated by the proportion of heterozygotes detected in the target plant, since all pollen source plants were fixed for an alternate allele. Variance of the outcrossing estimates was calculated following Brown, Matheson, and Eldridge (1975). All planned comparisons between types and populations within types were tested by an RXC test of independence using William's adjustment to the

G-test (Sokal and Rohlf 1981). Control plants were compared to the appropriate target plants using the G-test.

2.2.3 Floral biology

Like most members of the genus Solanum, eastern black nightshade flowers are presented in racemes in which multiple flowers are open concomitantly, allowing geitonogamous pollination (Whalen 1978). Pollen is dispersed from the anthers via terminal pores that are introrse. Insects, mainly bumblebees, extract pollen by "buzzing" the flower (Buchmann and Cane 1989), vibrating the pollen out of the pores in a stream.

Clusters of flowers from each family were sampled from two localities and preserved in FAA. Ten flowers from each plant were sampled to examine the following floral characters: 1) style, anther and filament lengths as estimates of flower size; 2) exsertion of the stigma beyond the anther tube (measured with an ocular micrometer); 3) style position (straight, curved or bent) and 4) number of anthers dehiscent. To assess the temporal dynamics of development, flowers were categorized into stages: 1) bud stage, 2) corolla fully opened, 3) corolla beginning to close, and 4) corolla closed. The pattern of anther dehiscence was used as an indirect measure of phenology. Population differences in the degree of stigma exsertion at stages 2 and 3 were tested by GLM procedure (PC-SAS 1988) after square root transformation to normalize the data and residuals. Population means were separated by Tukey's

Multiple Comparison Test (PC-SAS 1988). Floral measures were then correlated with familial estimates of outcrossing using Spearman's rank correlation (r_s).

2.3 Results

2.3.1 Outcrossing estimates

There was no difference in the level of outcrossing between the northern ruderals (P and R), and northern agrestals (W and H) when grown under agricultural conditions in Ontario. Both types had low levels of outcrossing (< 3%; Table 2.1), and could be pooled as a homogeneous northern group (G=1.09, df=3, p>0.05). The southern genotypes (NC and GA) had higher levels of outcrossing than the northern populations. The two southern populations represent a heterogeneous group (G=14.56, df=1, p<0.05) with the outcrossing levels of the NC population (17.2%) almost twice that of the GA populations (9.8%). Temporal patterns in outcrossing rates could not be tested due to the low number of outcrosses detected in northern populations.

The intrapopulation controls (FF-G2 families 2 and 5, Table 2.2) did not have significantly greater outcrossing rates than the target plants surrounded by Ontario SS genotypes (G=3.85, df=3, p>0.05). Therefore these outcrossing estimates do not appear to be biased by pollen incompatibility between the geographically distant populations.

Families within each of the northern populations had

Table 2.1 Estimates of proportion outcrossing (no. of heterozgotes per individuals sampled) and its variance, for each type (indicating Pgm-2 genotype) and population of EBN, as a function of harvest date. The number of arrays are in parentheses after the population

TYPE/ POP[a]	HARVEST DATE[b] 1	2	3	4	5	TOTAL	% OUTCROSS	S^2
NORTHERN RUDERAL (SS)								
R (3)	1/60	0/50	2/30	0/20	0/10	3/170	1.77	0.010
P (4)	2/100	0/80	4/80	1/50	0/10	7/320	2.19	0.007
					Total	10/490	2.04	0.004
NORTHERN AGRESTAL (SS)								
W (4)	0/40	0/40	0/60	6/80	2/30	8/250	3.20	0.012
H (4)	1/30	0/40	6/120	2/100	0/30	9/320	2.81	0.009
					Total	17/570	2.98	0.005
SOUTHERN AGRESTAL (FF)								
NC (4)	7/80	11/50	26/90	0/50	11/50	55/320	17.19	0.044
GA (5)	3/50	0/20	20/120	7/110	9/100	39/400	9.75	0.022

[a] Population designations: R=Rondeau; P=Pelee; W=Wright; H=Harrow; NC=North Carolina; GA= Georgia.

[b] Harvest date: 1=8/30; 2=9/5-9; 3=9/11-15; 4=9/25; 5=10/3-6

Table 2.2 Estimates of proportion outcrossing for individual families in each population of EBN

TYPE/ POP[a]	Pgm GENOTYPE	FAMILY	# of ARRAYS	TOTAL	MEAN % OUTCROSS
NORTHERN RUDERAL (NR)					
R	SS	1	2	1/90	1.11
	SS	4	2	2/80	2.50
P	SS	1	2	6/160	3.75
	SS	5	2	1/160	0.63
NORTHERN AGRESTAL (NA)					
W	SS	1	2	6/160	3.75
	SS	4	2	2/90	2.22
H	SS	1	2	4/160	2.50
	SS	3	2	5/160	3.13
SOUTHERN AGRESTAL (SA)					
NC	FF	1	2	31/160	19.37
	FF	6	2	24/160	15.00
G1	FF	2	1	9/80	11.25
G2	FF	2	2	13/160	8.13
	FF	5	2	17/160	10.63
	SS	4	1	1/80	1.25
CONTROL[b]					
G2	FF	2	1	10/90	11.11
	FF	5	1	14/80	17.50

[a] See Table 2.1 for population designations

[b] Control=FF target plants surrounded by SS-GA plants.

similar outcrossing rates (Table 2.2), but as expected frequencies within the outcrossed cells were less than 5, population homogeneity could not be evaluated. The two families from NC represent a homogeneous unit ($G=1.07$, $df=1$, $p>0.05$), but the GA populations showed a mixture of outcrossing types. The FF genotypes had a higher outcrossing rate than the SS genotype (8-11% vs 1%). Although accidental mowing of the second GA-SS array makes statistical comparison impossible, the results suggest that the GA populations are comprised of plants with variable outcrossing potentials.

The northern and southern types differ not only in the mean outcrossing levels, but also in the distribution (Table 2.3) of the outcrossing events among berries. Each target plant in the southern populations had a number of berries containing a mixture of self and outcross progeny but only two totally outcrossed berries occurred in one of the NC target plants. The elevated outcrossing rate was due to an overall increase in the number of heterozygotes per berry detected rather than a few flowers that received massive loads of outcrossed pollen.

All 30 individuals sampled from hand cross-pollinated berries of both FF and SS target plants were heterozygotes, suggesting that the stigma is receptive as the flower opens. The lack of outcrossing found in field experiments, when compared to the success of the hand crosses, suggested that outcrossing was precluded by morphology in northern plants.

Table 2.3. Comparison of between berry variability in number of heterozygous progeny detected per berry

TYPE*	POP	Pgm-2 GENOTYPE	NO. of HETEROZYGOTES/BERRY										
			0	1	2	3	4	5	6	7	8	9	10
NR	R	SS	15	1	1	0	0	0	0	0	0	0	0
	P	SS	25	7	0	0	0	0	0	0	0	0	0
	Pooled		40	8	1	0	0	0	0	0	0	0	0
NA	W	SS	22	0	2	0	1	0	0	0	0	0	0
	H	SS	24	8	0	0	0	0	0	0	0	0	0
	Pooled		46	8	2	0	1	0	0	0	0	0	0
SA	NC	FF	15	8	1	3	1	0	2	0	0	0	2
	GA	FF	22	8	5	1	2	1	0	1	0	0	0
	GA	SS	7	1	0	0	0	0	0	0	0	0	0

* See Table 2.2 for type designations.

2.3.2 Floral biology

The flowers are chasmogamous and availability of pollen at each stage was similar for all populations (Table 2.4). Immediately upon flower opening (stage 2), one anther is dehiscent and self pollen is available. Once a flower bud opens, both the style and filaments elongate. The style attains its maximum length by stage 2, but the filaments continue to elongate. Style length was difficult to measure in southern individuals with bent styles, and was not analyzed further. Anther length remains constant throughout the four developmental stages, so only length at stage 1 is presented (Table 2.4). There are no differences in anther size between populations, with the exception of the NC population, which was biased by a single family with extremely small flowers. If anther length can be used as an accurate predictor of flower size, as it can in related wild tomatoes (Rick et al. 1977), it appears that differences in outcrossing levels between populations were not related to flower size.

The degree of stigma exsertion beyond the anther tube is independent of flower size, and in other species has been shown to have a genetic basis (Rick, Holle, and Thorp 1978; Ennos 1981). Maximum exsertion occurred at stage 2 for all populations (Fig. 2.1), and ranged from 0.17-0.69 mm. Maximum stigma exsertion was significantly greater in southern populations (NC and GA) than in northern populations, if G2-4 (Pgm-SS) individual is excluded. There

Table 2.4. Means (SE) of # of anthers dehiscent at each developmental stage (1-4)[a], and anther length in the bud (stage 1), for each type and population of EBN. Style position is shown for each population

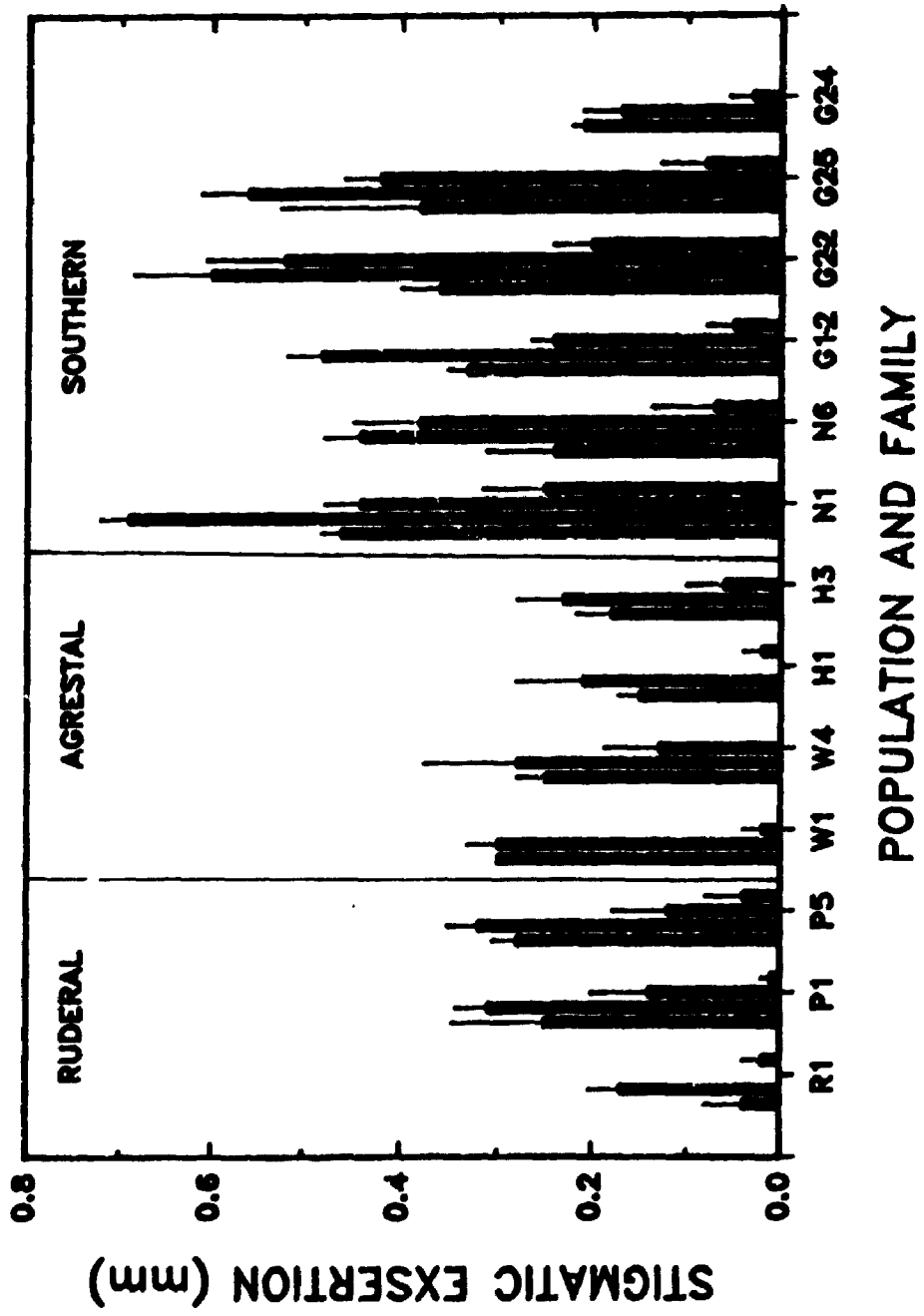
TYPE[b]	POP	# ANTHERS DEHISCENT				ANTHER LENGTH(mm)	STYLE POSITION
		1	2	3	4	1	
NR	R	0 (0)	1.0 (.63)	4.0 (.41)	4.4 (.25)	2.00 (0)	Straight
	P	0 (0)	1.2 (.25)	3.7 (.26)	4.9 (.11)	1.93 (.053)	Curved/Straight
NA	W	0 (0)	0.7 (.30)	2.8 (.42)	4.5 (.19)	1.91 (.049)	Curved/Straight
	H	0 (0)	0.9 (.32)	4.0 (.23)	4.8 (.20)	1.88 (.037)	Straight
SA	NC	0 (0)	0.9 (.25)	3.5 (.37)	5.0 (0)	1.7 (.069)	Bent
	GA	0 (0)	1.0 (.58)	3.7 (.27)	4.9 (.08)	1.94 (.027)	Bent
	GA[c]	0 (0)	0.6 (.26)	2.8 (.63)	4.8 (.25)	2.03 (.048)	Bent/Straight

[a] stage 1=bud; stage 2 and 3=fully opened; stage 4=corolla closed

[b] See Table 2.2 for type designations.

[c] Pgm-SS genotype separated from the above Pgm-FF genotypes.

Fig. 2.1. Mean (SE) stigmatic exsertion (mm) for each family of Solanum ptycanthum. Each of the 4 bars per family represents a developmental stage from bud (1) to corolla closure (4), grouped by type (northern ruderal, northern agrestal, and southern agrestal) and population.


STIGMATIC EXSERTION (mm)
0.0
0.2
0.4
0.6
0.8
RUDERAL
AGRESTAL
SOUTHERN
R1
P1
P5
W1
W4
H1
H3
N1
N6
G12
G22
G25
G24
POPULATION AND FAMILY

was no consistent pattern between northern ruderals and agrestals. Within the ruderal populations, P had individuals with a significantly greater degree of stigmatic exsertion than R individuals ($p<0.05$). The two agrestal populations (W and H) are intermediate to these ruderals. Within each of the northern populations, families showed little variability in exsertion or its developmental pattern (Fig. 2.1). The stigma depth is approximately 0.2 mm, so even at maximum exsertion, stigmatic surfaces of the northern populations are in very close proximity to the anther pores. Stigmas of southern plants also stay exserted significantly longer (stage 3) than those of northern plants (Fig. 2.1).

Style position (bent, curved or straight) also dictates when the stigmatic surface contacts the anthers (Table 2.4). Southern populations have bent styles which removes the stigmatic surface from the vicinity of the anther tube. This position precludes contact with self pollen until the filaments elongate to bring the anther pores into proximity of stigmatic surface just before the flower closes (stage 3). The northern populations may have a slightly curved style in the bud stage, but it very quickly straightens (by stage 2), so that the stigma is in direct line with the pore openings. Therefore, as the pollen sifts out of the anther sacs, due to insect visitation or some other motion (e.g., gravity), stigmas of the northern populations will be in direct contact with self pollen as soon as the flower opens.

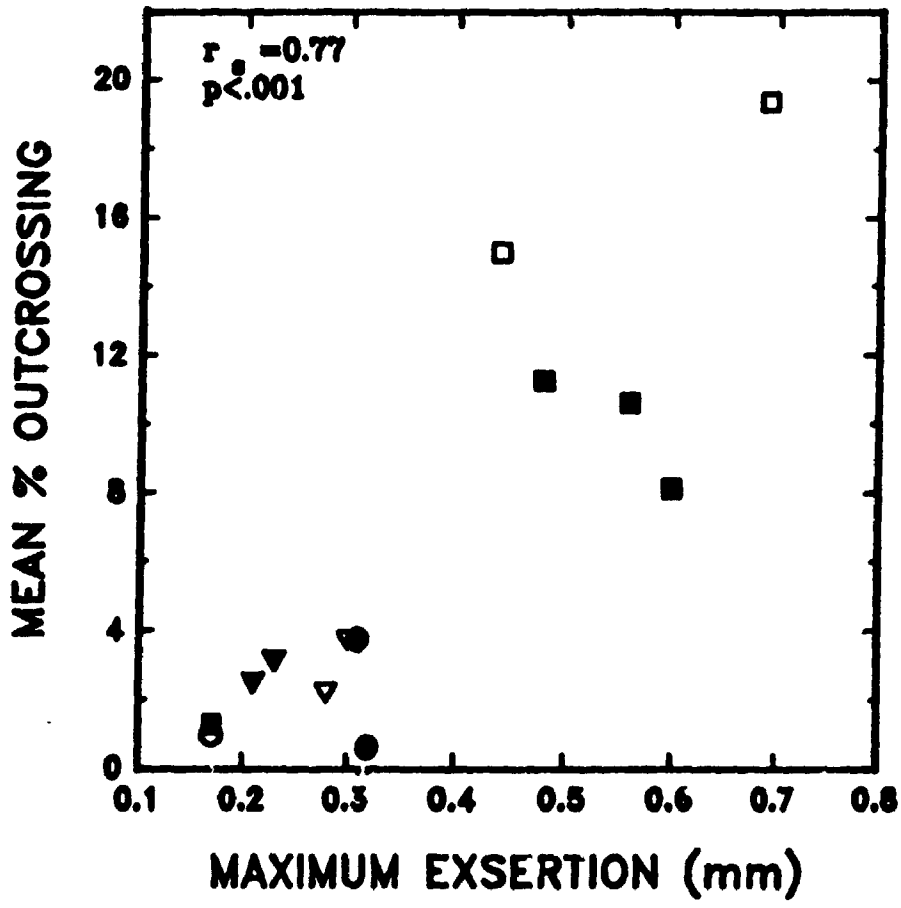
The total overlap in male and female functions permits self pollination immediately upon flower opening in northern populations while the window for receiving outcross pollen is much larger in southern populations.

The single SS plant from the G2 population (G2-4) had flowers with very limited stigma exsertion (Fig. 2.1). In addition, the position of the style is bent in the bud stage, but straightens out to contact the anther tube shortly after the flower opens. These differences are consistent with the low outcrossing rate found in the field. Although the GA population appeared to be polymorphic in floral structure, a continuous variation pattern could not be ruled out due to the limited number of families originally sampled.

2.3.3 Correlations between floral characters and outcrossing estimates

The amount of outcrossing in Solanum ptycanthum is significantly correlated with the maximum degree of stigma exsertion (r_s=0.77, $p<0.001$, df=13; Fig. 2.2). The GA Pgm-SS genotype falls within the distribution of northern genotypes, rather than with the more outcrossed southern group. There is a significant correlation with stigma exsertion at all stages, but the correlation with stage 2 (maximum exsertion) is the highest. Exsertion at stage two is highly correlated with exsertion at other stages (each $r_s>0.8$, $p<0.001$). Correlations between outcrossing and any other measure of flower size, at any stage were not

Fig. 2.2. Association between maximum stigmatic exsertion (i.e., at stage 2) for each family of Solanum ptycanthum, and mean % outcrossing (ruderal:R-○ P-● ; northern agrestal: H-▼ , W-▽ ; southern agrestal: GA-■ , NC-□).


r_s =0.77
p<.001
MEAN % OUTCROSSING
0
4
8
12
16
20
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
MAXIMUM EXSERTION (mm)

significant, with the exception of the length of the filament at stage 3. The rate of filament elongation would dictate how long the stigma stayed exserted beyond the anthers, and hence increase the length of time outcrossing may occur. The utility of stigma exsertion in the prediction of outcrossing rates in other species is variable. Exsertion is correlated with outcrossing rate in wild tomatoes (Rick et al. 1977; 1978) and Ipomoea (Ennos 1981), but not in Gilia (Schoen 1982) or Clarkia (Vasek and Harding 1976).

2.4 Discussion

Mating systems of colonizing plants are flexible, and examples of species which have demonstrated a decrease or increase in the amount of outcrossing in newly founded colonies have been documented (Brown and Marshall 1981). The breeding system of S. ptycanthum expressed significant flexibility when grown in agrestal habitats at the northern margin of its distribution. Genotypic variability in outcrossing ranged from 0.63-19% and was greater than expected for a weedy annual (e.g., Warwick 1990b). Marginal northern populations, both agrestal and ruderal, sampled from regions in which EBN is actively expanding its range, show low outcrossing potential compared to other colonizing ruderal species (Adams and Allard 1982; Jain 1975). Schilling (1978) obtained similarly low outcrossing estimates (<6%) in S. americanum, a closely related species,

but the habitat origins of the five individuals was not specified. Andersen (1988) found outcrossing varied from 0.7-4.2% in six agrestal families from a single population in Abutilon theophrasti, with a mean of 2.8%. This agrestal weed also displayed a similar floral morphology and phenology that restricted the opportunity for outcrossing (Andersen 1988). Andersson (1989b) suggested "field" (i.e., agrestal) populations of Crepis tectorum were more autogamous (i.e. set more seed) than "ruderal" populations, although actual estimates of outcrossing levels were not done.

In contrast, southern agrestal populations show relatively high outcrossing rates under experimental conditions in Ontario. It appears that the mating system of EBN has shifted toward decreased levels of outcrossing upon colonization. However, outcrossing levels of both the southern agrestal, and northern ruderal individuals may be over-, or under-estimated due to the presence of phenotypic plasticity in the expression of stigmatic length, when grown in northern agrestal environments. In a series of greenhouse experiments conducted over a wide range of environmental conditions, these same populations showed no gross shifts in stigmatic expression. This suggests that the observed outcrossing estimates are representative of levels found in "home" habitats in the south. Further study of actual outcrossing rates in southern localities would be necessary to verify this observation. Variability in

pollinator density may also modify t..e actual outcrossing rate.

Decreased outcrossing in geographically marginal populations has also been documented in Lycoperaicon (Rick et al. 1977), Gilia (Schoen 1982) and Eichornia (Glover and Barrett 1986). Outcrossing rates of northern (Ontario) populations of Carduus nutans (Warwick and Thompson 1989) were lower than southern (Kansas) populations (Smyth and Hamrick 1987). The rationale for this decrease has been based on the selection of selfing variants upon colonization of marginal habitats, and therefore reproductive assurance. This would not seem to be the case in S. ptycanthum, as reproductive output is high in all populations, regardless of outcrossing rate (Appendix C). Other selectively important characters may differentiate the various founders.

Outcrossing levels estimated in these simulated field conditions probably represent the upper limit of outcrossing potential. The absence of filtering effects generated by the presence of other species (Campbell 1985) and uniformly high densities ensured maximum outcrossing (Smyth and Hamrick 1987; Golenberg 1988). Genotypic differences in pollen availability may increase the outcrossing rate in selfing species (Golenberg 1988). Although it is impossible to determine if each array had an equal probability of receiving outcross pollen, the lack of correlation between anther size and outcrossing, the similar pattern of anther dehiscence, and equal anther sizes (i.e., similar pollen

volume) between allozyme genotypes minimized any bias which may be linked with the use of the "southern" F allele.

Gene flow via pollen in predominantly selfed agrestal species may be low, but agricultural machinery redistributes seeds throughout fields (populations) (McCanny and Cavers 1988), which decreases the probability of sib matings and increases effective outcrossing (Schemske and Lande 1985). This anthropogenic movement of seeds also ensures continuous invasion of new areas, and recolonization of old habitats.

The occurrence of a "non-exserted" phenotype within the GA populations suggests that the northern populations may represent a subset of this population, and may have been founded by the highly selfed line derived from Georgia populations. The ubiquitous distribution of the Pgm-S allele in Ontario is consistent with this possibility. In the past, the direct input of the southern genotypes into Ontario via the yearly importation of tomato transplants from Georgia would have provided such an avenue of invasion. However further research on the distribution of floral phenotype and the correlation with allozyme genotype across nightshade's range would be needed to verify this possibility.

The absence of suitable genetic markers in predominantly autogamous species has prevented detailed investigations of breeding system variability of annual, weedy species. Marginal, northern populations of S. ptycanthum, both ruderal and agrestal, conformed to

expectations of low levels of outcrossing. However, the role of multiple introductions of founders over time in determining potential genetic variability of colonizing weed populations must also be evaluated (Barrett 1988), especially since these founders often differ in quantitative traits affecting survival and reproductive success (Allard et al. 1968).

CHAPTER THREE--TEMPERATURE-DEPENDENT GERMINATION

3.1 Introduction

The invasion success of a colonizing weed depends upon the ability of its seed to germinate in new habitats (Groves 1986). Temperature regime constitutes one of the most potent selective forces limiting the spread of weeds (Lindsay 1953; Thompson 1970; Woodward 1987). Extreme temperatures may kill the seeds outright, or temperature fluctuations may be such that seedlings emerge at inappropriate times, and suffer massive mortality. Many weedy species exhibit the potential to germinate over a wide temperature range (Cumming 1959; Grime et al. 1981; Weaver and Thomas 1986; Wagner 1988), facilitating potential range expansion (Groves and Kaye 1989). But significant variability in germination profiles has also been documented both among (McKell et al. 1962; McWilliams et al. 1968; Frost and Cavers 1975; Jain 1982; Naylor and Abdalla 1982; Warwick and Black 1986), and within (Cavers and Harper 1966; Palmblad 1969; Wulff 1973; Jain 1982; Weaver et al. 1985; Wulff 1988) populations of weedy species. An evolutionary interpretation of germination studies is often limited (Baskin and Baskin 1973; Quinn and Colosi 1977), because the genetic component of germination character variation cannot be separated from environmentally induced variation (but see McKell et al. 1962; McWilliams et al. 1968). In addition to genetic variability in germination characters, phenotypic

plasticity (Sultan 1987) may also play an integral part in colonization potential (Blais and Lechowicz 1989), via accommodation by individual genotypes to a novel range of temperatures.

During the last century in North America there has been a northward migration of many weeds of southern origin (Warwick 1990b), although the directness of the migration routes is often unclear. Northern populations of these migrants sampled from the geographical margins of the species' ranges show substantial life history variability, both among and within populations (Weaver et al. 1985; Warwick 1990b), but comparison to "central" populations is lacking. The selective environments of southern and marginal northern populations of weedy species are potentially different, especially for factors related to climate such as temperature regime.

Most weeds have wide ecological amplitudes (Baker 1974; Brown and Marshall 1981), and successfully colonize both agrestal and ruderal habitats. Agricultural habitats are selectively different than ruderal habitats (Snaydon 1980; Warwick 1990b), and adjustments in emergence time to optimize survival would be expected (Barrett 1983). For example, delayed germination in agroecosystems would minimize seedling mortality due to pre- and post-planting herbicides, and spring ploughing, while in ruderal habitats germination early in the season ensures ample moisture and nutrients for seedlings. Therefore, thermal cues to

initiate the germination response and/or the speed with which it proceeds may reflect the differences between ruderal and agrestal habitats. Agroecosystems are also thought to be environmentally more homogeneous when compared to ruderal habitats (Barrett 1982), suggesting that germination behaviour of agrestal populations may be genetically less variable than ruderal population. The role played by plasticity in the potential colonization of such divergent habitats is unknown.

To assess colonization potential, I studied the relative amounts of inter- and intrapopulation genetic differentiation and plasticity in the germination response to temperature of northern ruderal and southern agrestal populations of eastern black nightshade, and compared both to the recently established northern agrestal populations. Seed weight variability and its correlation with germination characters is also documented.

3.2 Materials and Methods

3.2.1 Background

Five familiies (or 3, in G1) from each population were selected (see section 1.7). Seeds were extracted from berries in August 1989 as described in section 1.7, and stored in paper envelopes at 4°C until use. Eastern black nightshade has an indeterminate growth pattern, with axial inflorescences borne at dichotomous branch points. Berries from different developmental positions were pooled. Only

seeds which appeared fully formed upon visual inspection were included in the following experiments.

3.2.2 Germination response to temperature

Germination tests were conducted over a range of temperatures using three replicates of 100 seeds per family at each temperature. Seeds were placed in 9 cm glass Petri plates on Whatman #3 filter paper and moistened with 10 ml of distilled water. Additional water was added as needed. At the lowest temperature, 0.5% streptomycin solution was used to discourage fungal growth. The incubator was set at alternating day/night temperatures of 40/30, 35/25, 30/20, 25/15, 20/10 or 18/8 °C, with a 14 hr day, giving mean daily temperatures of 35.8, 30.8, 25.8, 20.8, 15.8, and 13.8 °C, respectively. Light is required for germination except at high temperatures, and optimal germination requires alternating temperatures (Thomson and Witt 1987). A 10°C daily fluctuation in temperature is typical during peak emergence in May and June in southwestern Ontario (Weaver et al. 1988). Temperature tests were run sequentially due to space limitations but in haphazard order (25.8, 30.8, 15.8, 20.8, 35.8, 13.8).

The confounding effects of seed aging over the time period of the experiments (August, 1988 to May, 1989), was measured by re-running the 30.8°C germination test in August 1989 and comparing results to the original run.

Light levels (photosynthetically active radiation, 400-700 nm) were measured at the start of each temperature run

and fluorescent bulbs replaced when necessary. Light levels were not significantly different between runs ($F_{[5,85]}$=0.5, p=0.72), and ranged from 27.7-30.1 μmol $m^{-2}s^{-1}$. Within the incubator, Petri plate positions were rotated daily. Germinated seeds were counted and removed every 12 hours at the two highest temperatures, and daily at the other temperatures. Duration of each run varied, from <25 to 60-70 days for high and low temperatures, respectively. Runs with less than 100% germination were terminated when germinations had ceased for a period of 1-2 weeks. All seeds were considered initially viable, as each seed lot germinated to 100% in the second 30.8°C run. To test if the ungerminated fraction remained viable or had entered dormancy at the termination of each run, seeds were transferred to optimal temperature (30/20°C), and those which failed to germinate within 3 days were dissected in 0.5% tetrazolium solution and viability scored after 24 hours.

To test within and among population differences in seed weight, and its correlation to germination parameters, four lots of 10 seeds were weighed from each family in January 1989, to the nearest hundredth milligram with a Cahn 4700 electrobalance.

3.2.3 Statistical analysis

Interpopulation differences in germination speed (T_{50}: time taken to reach 50% germination) and the final % germination in response to temperature were compared with a

2 way mixed model ANOVA (Type III sum of squares due to missing values in T_{50}), with population (random) and temperature (fixed) as main factors (Proc GLM; PC-SAS 1988). In this model intrapopulation variation was included in the error term. Random effects (population and interaction) used the error MS, and fixed effect (temperature) used the interaction MS as the denominator for significance tests (Sokal and Rohlf 1981). At each temperature, a nested ANOVA and the following contrasts were performed using family nested within population as the error term: differences between populations within each type, and differences between types, pooling appropriate populations. Differences among population means across all treatments were tested by a nested ANOVA, followed by contrasts specified above. The relative amount of variability accounted for by population and family components at each temperature was apportioned by Proc NESTED (PC-SAS 1988).

Intrapopulation variability for the germination characters was compared with a similar ANOVA, but with the family component designated as random. In this design the family component pools additive and non-additive sources of genetic variation, as well as genetic maternal effects, and the error term (within family) encompasses random environmental differences, residual non-genetic maternal effects, and any positional effects of the berries within the maternal plant. A significant family component indicates among family differences in overall mean response

for each trait. A significant environmental component indicates the presence of plasticity. The genetic basis of plasticity is measured as the family by environment interaction. Norm of reaction diagrams (Sultan 1987) accompany these ANOVAs to illustrate variability among families in response to temperature in each population.

Final % germination was arcsin transformed and T_{50} was log transformed to normalize residuals (Proc UNIVARIATE; PC-SAS 1988) and stabilize variances (Barlett's test for the homogeneity of variance) in all above ANOVAs. The lowest temperature level (13.8°C) tested was not included in any of the ANOVA's due to the lack of germination in many of the families.

The amount of plasticity of each population was compared by the mean coefficient of variation (CV) of families measured across all temperatures (Schlichting and Levin 1984), and population differentiation assessed by the Kruskal-Wallis test (Proc NPAR1WAY; PC-SAS 1988).

Seed weight variability among families and populations was tested by a nested ANOVA (Proc GLM) using untransformed data. Contrasts were specified as above. Spearman rank correlation coefficients were used to determine the association between seed weight and the germination speed and extent across all populations.

Another important component of the germination syndrome which determines colonization potential is the base temperature. This is the threshold temperature below which

no germination occurs, and was calculated as:

$$[1] \qquad \frac{1}{T_{50}} = \frac{T - T_b}{\theta}$$

where T_{50} is the time to 50% germination, T is the actual temperature, T_b is the base temperature, and θ is the thermal time (degree days, °Cd) required for 50% germination (Garcia-Huidobro et al. 1982). The reciprocal of T_{50}, or rate, increases linearly as a function of temperature to a maximum value at the optimal temperature, beyond which rate decreases. Extrapolation to the x intercept (i.e., where rate=0) in the linear sub-optimal portion of the function estimates T_b. For each population, mean familial rate was regressed against temperature over the linear portion of the curve (excluding 13.8 and 35.8°C). The base temperature was estimated from $T_b = -b_0/b_1$, where b_0=intercept and b_1=slope, and thermal time to 50% germination was estimated as the inverse of the slope (Hsu et al. 1984). Nonparametric tests (Proc NPAR1WAY) were used to discriminate populations.

3.3 Results

3.3.1 Effects of seed age

There were no changes due to seed aging in final % germination in any population (each population $F_{[1,28]}<1.00$, $p>0.05$). Time taken to reach 50% germination increased significantly in the Rondeau ($F_{[1,28]}=5.78$, $p=0.0230$) and Southern (G1:$F_{[1,16]}=68.37$, $p<0.0001$; G2:$F_{[1,23]}=15.80$,

p=0.0004) populations, but by less than half a day. Compared with the magnitude of observed responses, such small changes in germination potential indicate that the ageing effect was minimal, and it was disregarded in the following analyses.

3.3.2 Germination response to temperature

Speed and extent of germination were highly temperature dependent (Figs. 3.1-3.3). Both germination traits showed significant variation among and within populations (Tables 3.1, 3.2). In most cases the total cumulative germination was greater than 90% over a range of mean daily temperatures from 20.8-30.8°C, which delineates the optimal range. Final germination declined abruptly at lower or higher mean temperatures. The number of days required to reach 50% germination decreased with increasing temperatures to a minimum at the optimal temperature of 30.8°C, and then increased at the supra-optimal temperature of 35.8°C. Seeds derived from greenhouse-grown individuals varied among and within populations in weight (Fig. 3.4).

3.3.2.1 Variation among populations

The two populations within each of the northern (NA) and southern (SA) agrestal types were similar over all temperatures and were pooled for comparison (all $F_{(1,22)}<3.0$, $p>0.05$). At the lowest temperature SA populations germinated to a greater extent (80.1 $\pm$ 5.2% vs 53.6 $\pm$ 6.0%: $F_{(1,22)}=4.5$, $p=0.04$), but more slowly (41.6 + 3.2 days vs 23.9$\pm$ 1.1 days: $F_{(1,22)}=6.4$, $p=0.02$) than the NA type (Fig.

Fig. 3.1 Population response curves of EBN for final % germination and germination speed as a function of mean temperature. Note that 13.8 °C is included in only the final % germination.

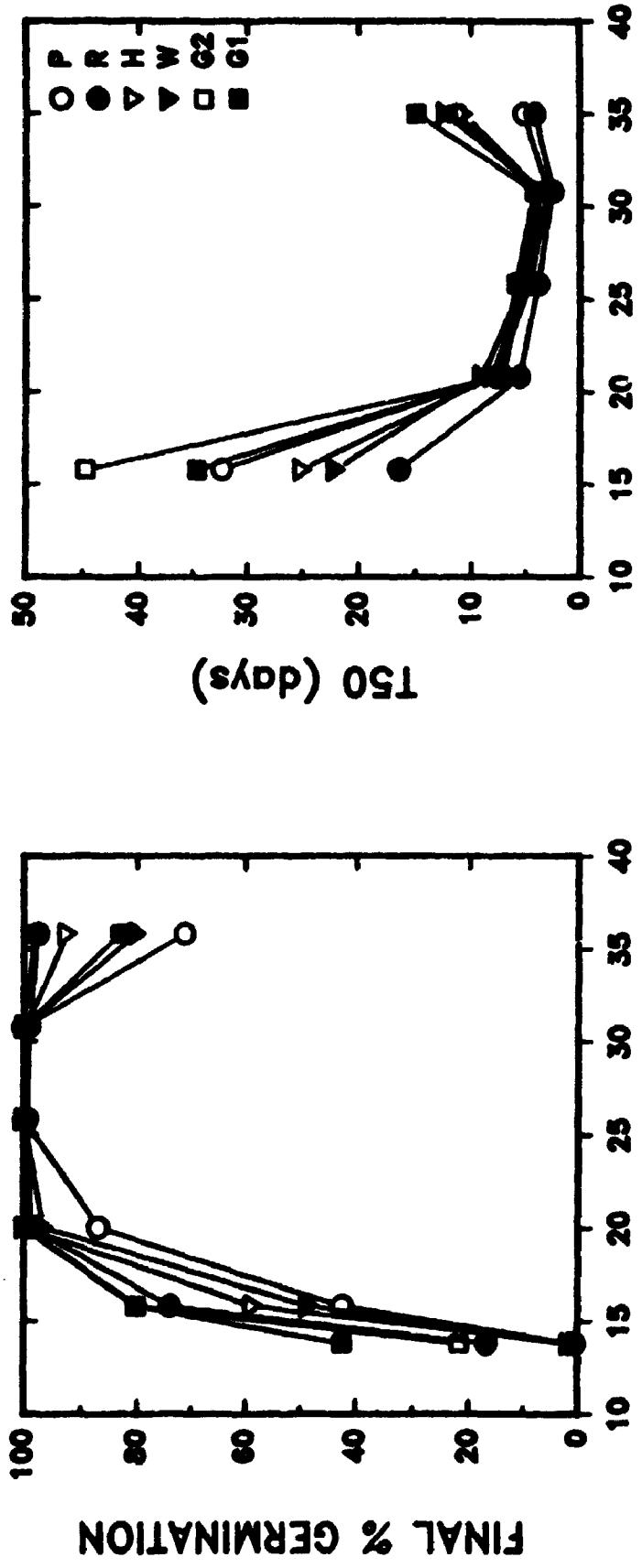

P
R
H
W
G2
G1
T50 (days)
FINAL % GERMINATION
MEAN TEMPERATURE (° C)

Fig. 3.2 Norm of reaction plots of each population of EBN to show variation among five (or three for G1) families for final % germination as a function of mean temperature. Note that 13.8°C is included in this figure, but not in the ANOVA. Symbols denote individual families.

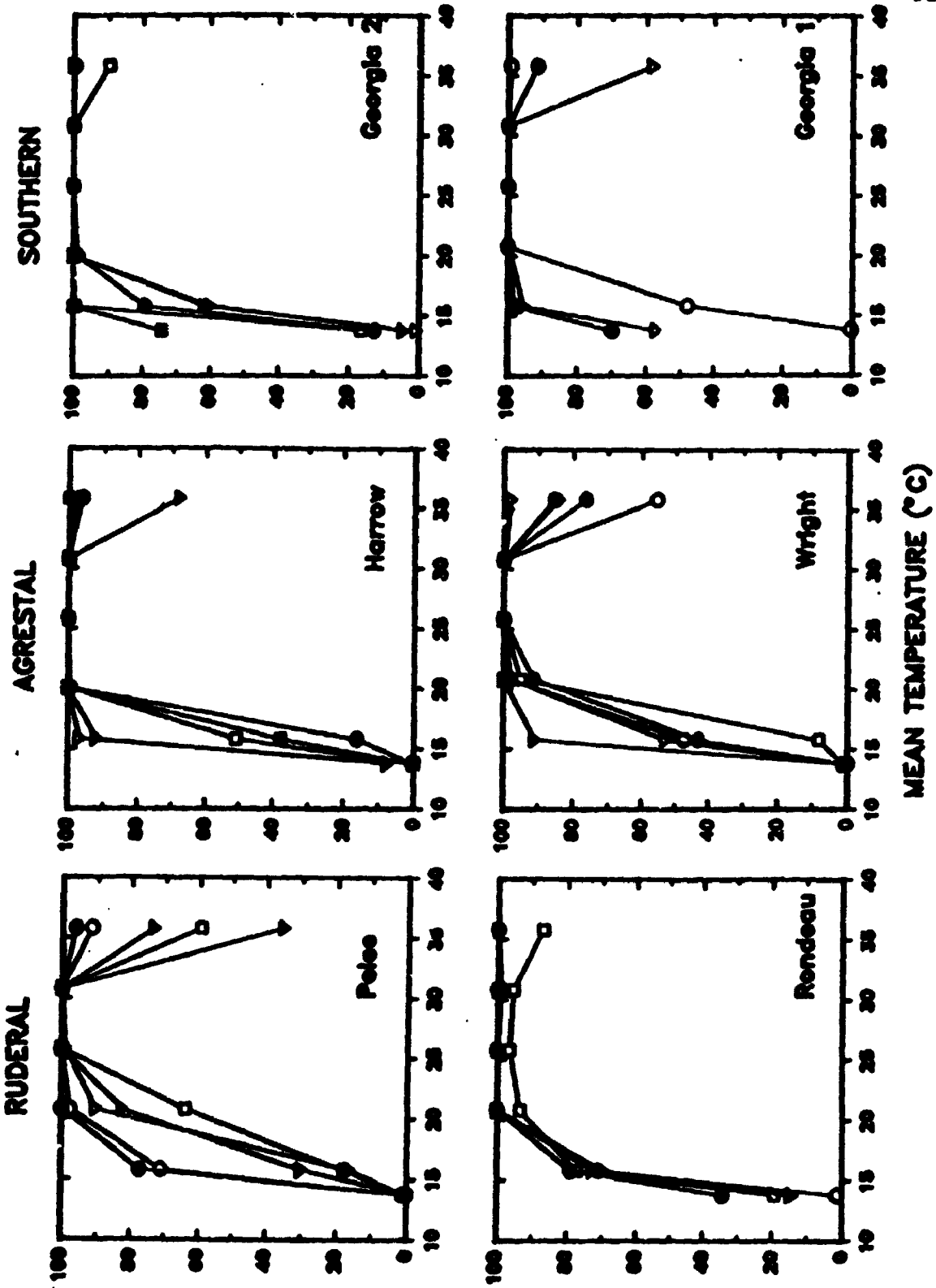

SOUTHERN
AGRESTAL
RUDERAL
Georgia 2
Georgia 1
Harrow
Wright
Pelee
Rondeau
FINAL % GERMINATION
MEAN TEMPERATURE (°C)

Fig. 3.3 Norm of reaction plots of each population of EBN to show variation among families for germination speed (T_{50}) as a function of mean temperature. Note the difference in the y axis in G2 population, and missing values at 15.8°C (P, H, W, and G1).

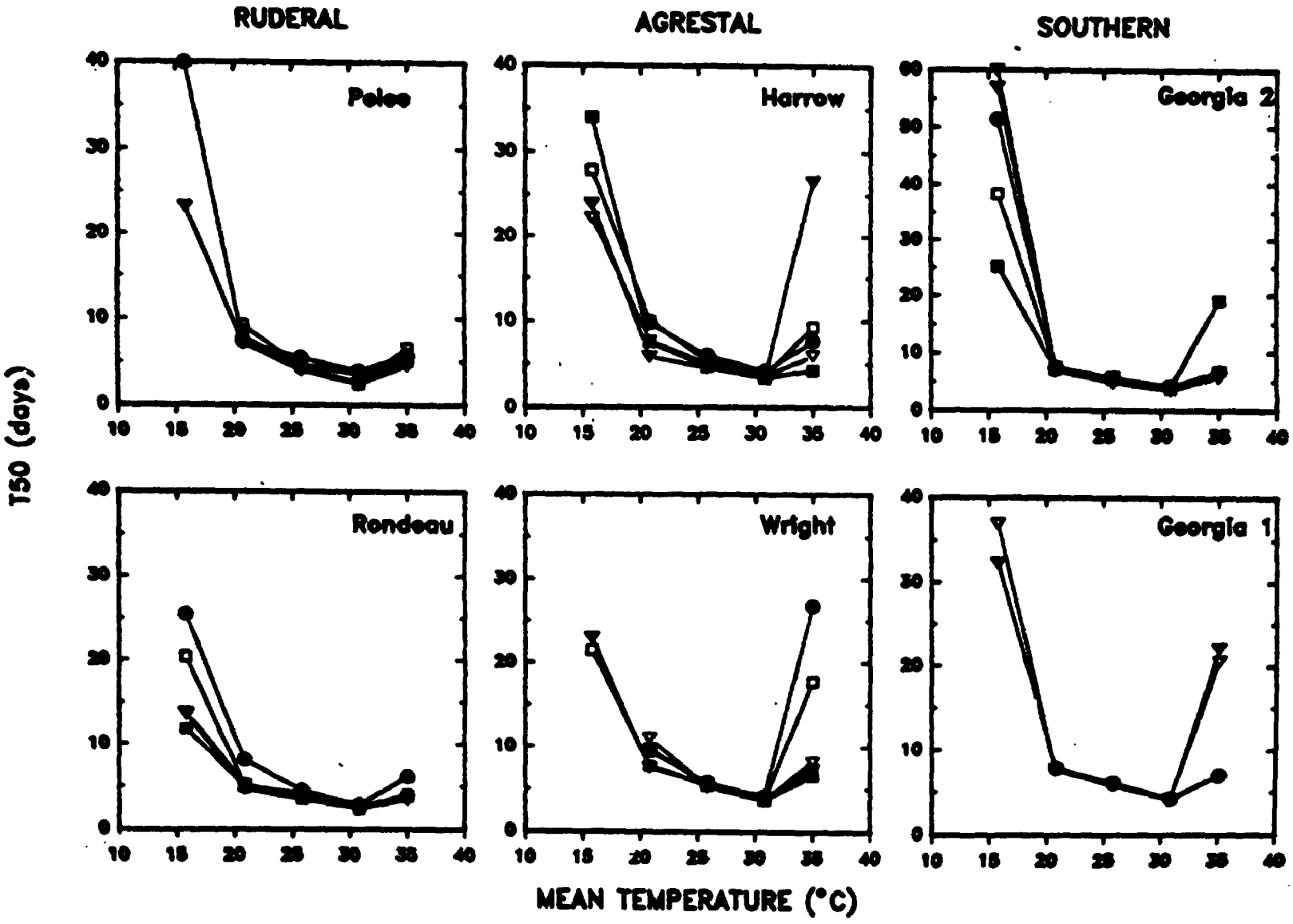

RUDERAL
AGRESTAL
SOUTHERN
Pelee
Harrow
Georgia 2
Rondeau
Wright
Georgia 1
T50 (days)
MEAN TEMPERATURE (°C)

Table 3.1 ANOVA results for final % germination and T_{50}, among the six populations of EBN in response to temperature

SOURCE OF	% Germination			T_{50}		
VARIATION	df	MS	F*	df	MS	F*
Temperature	4	4.94	28.96	4	6.17	86.96
Population	5	0.63	15.15	5	0.68	49.31
T x P	20	0.17	4.09	20	0.07	5.13
Error	390	0.04		351	0.01	
Total	419			380		

* $P < 0.0001$ for all F values

Table 3.2 Mean square values of each population for final % germination (A), and T_{50} (B), of EBN in response to temperature, and the significance of the F ratios. (All F ratios are significant at P < 0.001 unless otherwise noted)

SOURCE OF VARIATION	df	TYPE/POPULATION NR Pelee	NR Rondeau	NA Harrow	NA Wright	SA G2	SA G1
A.							
Temp.	4	1.947	0.650	1.139	1.729	0.387*	0.338ns
Family	4 +	0.436	0.111	0.120	0.151	0.047*	0.038ns
T x F	16 #	0.101	0.014ns	0.161	0.100	0.082	0.163
Error	50 %	0.021	0.009	0.015	0.011	0.014	0.015
B.							
Temp.	4	1.026	1.292	0.926	0.759	2.213	0.795
Family	4 +	0.033	0.089	0.036	0.053	0.015	0.050
T x F	&	0.012	0.007	0.071	0.048	0.065	0.045
Error	@	0.002	0.003	0.001	0.003	0.001	0.002

* P < 0.02 ; ns=non-significant

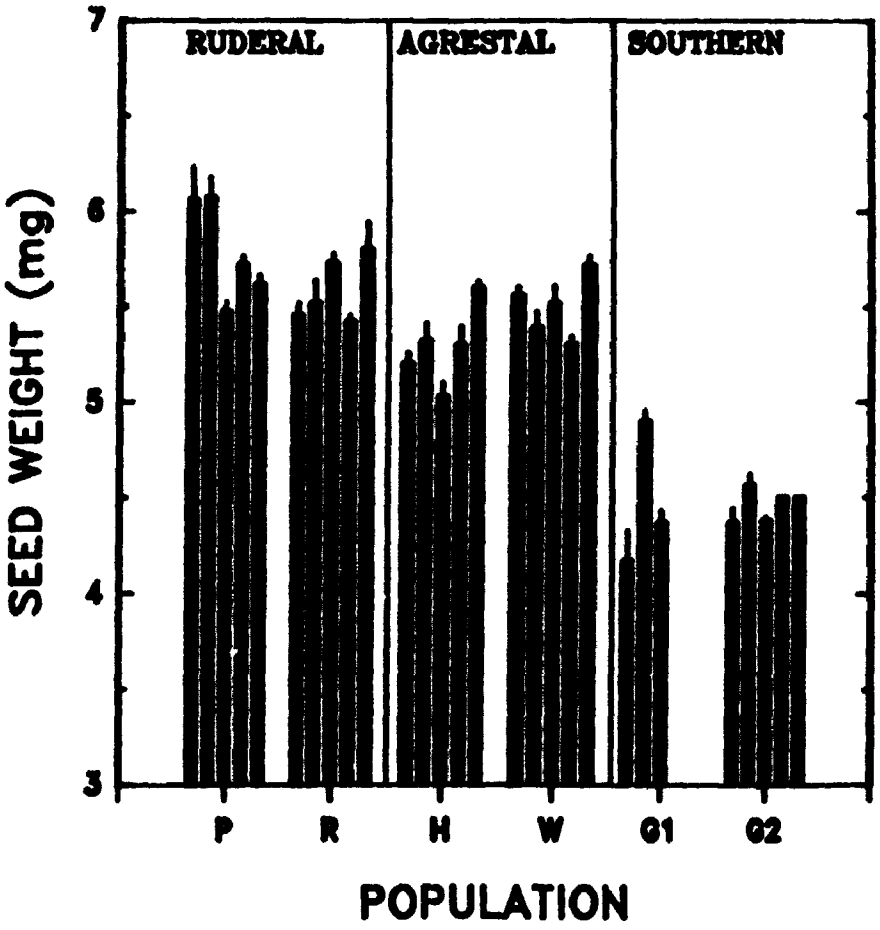
\+ Family df=2 for G1; # Interaction df=8 for G1;

% Error df=30 for G1.

& Interaction df=16 for R, G2; 15 for H; 13 for W, P; 7 for G1

@ Error df=49 for R; 48 for G2; 44 for H; 42 for W; 40 for P; 26 for G1

Fig. 3.4 Mean weight (± SE) of 10 seeds of EBN raised under greenhouse conditions for one generation. The bars represent individual families within northern ruderal (Pelee and Rondeau), northern agrestal (Harrow and Wright) and southern agrestal (G1 and G2) types.


RUDERAL
AGRESTAL
SOUTHERN
SEED WEIGHT (mg)
7
6
5
4
3
P
R
H
W
G1
G2
POPULATION

3.1). At all other temperatures, NA and SA populations showed no difference in germination speed or final % germination (all $F_{[1,22]}<2.9$, $p>0.10$). Estimated base temperatures (T_b) and degree days to 50% germination (θ) were similar for the two types (Table 3.3). Both northern ruderal and agrestal populations produced heavier seeds than southern agrestal populations (5.55 $\pm$ 0.03 mg vs 4.54 $\pm$ 0.05 mg: $F_{[1,9]}=77.2$, $p<0.0001$)(Fig. 3.4).

The germination profiles of the two ruderal populations, Pelee and Rondeau differed (Fig. 3.1). Seeds from the Rondeau population germinated faster, and to a greater extent than those from the Pelee population at all temperatures (all $F_{[1,22]}>4.3$, $p<0.05$), with one exception: T_{50} at 35.8°C ($F_{[1,22]}=0.6$, $p>0.05$). Seeds from NA populations germinated more slowly than those from Rondeau at all temperatures (all $F_{[1,22]}>4.8$, $p<0.05$), but germinated more slowly than those from the Pelee population only at 25.8 and 30.8°C ($F_{[1,22]}>8.2$, $p<0.009$). Averaged over all temperatures, the speed of germination was slower in both agrestal types when compared to the ruderal type (NR vs NA: $F_{[1,22]}=11.4$, $p=0.003$; NR vs SA: $F_{[1,22]}=26.2$, $p=0.0001$). Northern ruderal and agrestal populations did not differ in final % germination at any temperature (NR vs NA: $F_{[1,22]}<2.86$, $p>0.10$). Although estimated T_b did not differ between habitat types, the number of degree days to 50% germination was significantly lower in the ruderal populations than in the agrestal populations (Table 3.3).

Table 3.3 Population means for EBN of base temperature (T_b) and degree days to reach 50% germination (Θ). Probability values based on Mann-Whitney U test for type differences

TYPE	Population	T_b * (°C)	Θ * (°Cd)
NORTHERN RUDERAL (NR)			
	Pelee	14.4	48.5
	Rondeau	12.6	46.7
NORTHERN AGRESTAL (NA)			
	Harrow	13.0	64.4
	Wright	12.8	69.8
SOUTHERN AGRESTAL (SA)			
	G2	13.1	67.2
	G1	12.6	75.7

* NA vs SA, P=0.31; NA vs NR, P=0.65

* NA vs SA, P=0.08; NA vs NR, P=0.003;

Within northern populations, seeds from ruderal populations were heavier than those from agrestal populations (5.69 ± 0.04 mg vs 5.40 ± 0.036 mg: $F_{[1,22]}=9.2$, $p<0.006$), although Rondeau and Harrow populations are not significantly different.

In all six populations, the coefficient of variation of seed weight was <8%. Within each population type, seed weight did not differ between component populations (all pairs of populations $F_{[1,22]}<2.3$, $p>0.15$). Across all populations, mean family seed weight was negatively correlated with time to reach 50 % germination ($r_s>-0.56$, $p<0.001$) at all but one temperature (20.8°C), but seed weight was not correlated with total % germination. The increased low temperature germination rate of the northern agrestal populations may be a result of their larger seed weight. There were however, no significant correlations between seed weight and thermal time to 50% germination, or base temperature.

Population x temperature interactions were significant for both traits (Table 3.1), but relative magnitude rather than direction of response differed. Unlike the observed population differentiation found among trait means, there was no evidence for such differentiation in amount of plasticity for either trait (Table 3.4), reinforcing the finding that the genetic component of variability was more important than plasticity (population x temperature). The amount of plasticity was greater with respect to the speed

Table 3.4 Amount of plasticity in final % germination and T_{50}, measured by the CV (SE), for each population of EBN. Probability values based on Kruskal-Wallis test for population differentiation

TYPE/ Population	Coefficient of Variation (SE)	
	% Germination	T_{50}
NORTHERN RUDERAL		
Pelee	33.9 (9.04)	71.7 (16.37)
Rondeau	11.9 (1.10)	87.1 (5.70)
NORTHERN AGRESTAL		
Harrow	23.6 (7.37)	83.0 (31.00)
Wright	28.6 (7.00)	65.8 (9.81)
SOUTHERN AGRESTAL		
G2	10.4 (3.83)	121.1 (14.25)
G1	16.7 (6.67)	67.2 (21.48)
	p=0.16	p=0.17

of germination than final extent for all populations, reflecting the large optimal range of potential germination.

3.3.2.2 Variation within populations

Genetic differences among families in extent and speed of germination were significant in all populations (Table 3.2). The greatest intra-population genetic variability occurred at the extreme low and/or high temperatures (Figs. 3.2, 3.3). Southern and Rondeau populations exhibited the broadest temperature tolerance, with several families able to germinate at a mean daily temperature of 13.8°C (Fig. 3.2), which was very close to the estimated base temperatures of these populations (Table 3.3). Although all families did initiate germination, several did not reach 50% germination at 15.8°C (Fig. 3.2). At 35.8°C, time to reach 50% germination was most variable among families in agrestal populations (CV=50-80) compared to the ruderal populations (CV=20,21))Fig. 3.2). Seeds derived from individual families within each population were genetically different in average weight ($F_{[22,111]}=7.8$, $p<0.0001$) (Fig. 3.4).

The family x temperature interactions were significant for all populations, with one exception (Table 3.2). Several families within each population germinated to a greater extent (Fig. 3.2) and/or faster (Fig. 3.3) than the others at all temperatures, indicating differences in the relative magnitude of response generated the observed interaction rather than differential temperature-dependent selection (Antonovics *et al*. 1988; Ayres and Thomas 1990).

Intra- rather than inter-population differences accounted for a greater proportion of the total variability in extent of germination over the entire temperature range (Table 3.5). In contrast, differences among the populations accounted for a higher proportion of the total variability in germination speed at three of the five temperatures. The majority (80%) of the variance in seed weight was accounted for by among population differences. But regardless of population differences, the among family variability within all populations was significant.

3.4 Discussion

This study suggests that temperature would not present a barrier to the potential invasion of northern agricultural habitats by southern agrestal populations of S. ptycanthum. Seeds from southern populations were able to germinate over a broader temperature range than seeds from northern agrestal populations, and had a similar rate of germination at all but the lowest temperature. Estimated base temperature for germination did not vary between the southern and northern populations. In addition, minimum temperatures experienced in the winter do not restrict potential range expansion, as seeds of S. ptycanthum from southern populations can successfully overwinter in southern Ontario (Chapter 1).

Individual seeds from southern populations weighed less than those from northern populations, but seed weight was

Table 3.5 Percentage variance accounted for, and the significance of the F ratios among populations, and among families nested within population, for each temperature for the final % germination (A) and T_{50} (B). (All F values significant at P < 0.001, unless otherwise noted; df=5,22 except at 15.8 where df=5,14)

SOURCE OF VARIATION	MEAN TEMPERATURE (°C)				
	15.8	20.8	25.8	30.8	35.8
A.					
Population	10.5ns	34.7*	0.0ns	5.7ns	28.5*
Family(Pop)	59.6	48.8	68.5	60.9	47.4
R^2	0.81	0.88	0.77	0.77	0.83
B.					
Population	57.6	46.7	70.1	71.8	31.2
Family(Pop)	25.6	45.6	23.6	25.5	64.1
R^2	0.88	0.94	0.95	0.98	0.97

* P < 0.01; ns=non-significant

not strongly correlated with either percent germination or germination rate among the agrestal populations. Populations of widespread weedy species sampled over large latitudinal ranges show clinal response in germination traits and seed weight (McWilliams et al. 1968; Warwick and Black 1986), but many of these studies confounded seed size and/or phenotypic maternal effects within these findings (e.g., Palmblad 1969). Northern agrestal populations germinated faster at the low temperature, but this response is confounded with seed weight. Rapid divergence to a larger seed size in response to temperature may be possible, given the observed genetic variability within the southern population in seed size.

Within agrestal habitats, eastern black nightshade is a poor competitor. The high base temperature and greater thermal times found in southern agrestal populations ensures that nightshade will emerge after competitively superior weeds have been eradicated by herbicides (Weaver et al. 1988). Such germination characteristics would make northern agricultural habitats invasible by "preadapted" (Bazzaz 1986) agrestal genotypes from the south. Based on germination profiles, it is entirely possible that gene flow via importation of seeds in crates of tomato transplants, continually reintroduces southern genotypes into northern agrestal populations. Differences in other crucial life history characters, such as seedling growth rates or time to flowering may limit the invasive potential of the southern

populations.

Germination rate is an important fitness component (Jain 1982). Comparisons of northern ruderal and agrestal populations indicated that seeds from ruderal populations required approximately 45 degree days to reach 50% germination, while seeds produced by agrestals required 65-75 degree days. This difference in accumulated degree days is equivalent to 2-5 calendar days in early May in southern Ontario (30-yr. weather records, Harrow Research Station). Differences in emergence times may actually be larger, as determination of thermal times are underestimated in non-soil conditions, by as much as 50% (Weaver et al. 1988). Although Barrett (1983) has suggested delayed emergence may evolve in response to postemergence herbicide application, the use of soil applied herbicides which stay active in the soil for up to 6 weeks, along with the unpredictability associated with timing of spring cultivation and crop rotation, argues against such directional selection in most agrestal species. But tolerance of EBN to many of the herbicides approved for use in tomatoes may override some of these forces and allow potential divergence between agrestal and ruderal types. Optimal emergence time is also constrained by length of growing season, and crop growth dynamics of individual crops. Few examples of the formation of "agroecotypes" in response to agricultural practises have been documented, but many of these are restricted to the specific regimes under which they evolved (Barrett 1983;

Warwick 1990a). It is unknown if the amount of genetic variability and/or plasticity of germination traits found in E:N populations which grow in tomatoes would be ample to colonize other crops, such as soybeans which involve totally different suites of cultural practises. Different cultivation regimes are known to select alternate dormancy strategies (Jana and Thai 1987). Initial colonization of tomato crops may have constituted primary introduction foci, from which other types of crops were invaded (Bazzaz 1984).

Over the temperature range tested, agrestal populations did not show reduced levels of variability, either genetic or plastic, compared to ruderal populations. Nor did the geographically marginal northern populations show decreased levels of variability, compared to the central populations. Lack of observed differences may be due to the limited number of families sampled, in relation to population size. Differences found between the ruderal populations, compared to the similarity of the populations representing both agrestal types, points to the similarity of selective regimes among sampled agricultural habitats and/or colonizing genotypes. Genetic differences found between the two ruderal populations may be due to founder effect and/or genetic drift (Brown and Marshall 1981), both accentuated by the predominately self-pollinating nature of S. ptycanthum (<3% outcrossing; Chapter 2), or environmental differences between the two locations.

As expected, both germination characters were plastic

in response to temperature for all populations, but also showed differentiation in trait means. Plasticity has not acted to buffer the mean response from potential directional selection (Schlichting 1986; Sultan 1987; MacDonald and Chinnappa 1989). Comparison of ruderal and natural populations of the annual weed, Xanthium strumarium showed that small, coordinated differences in plastic responses of many life history traits differentiated these populations, although ruderal (more variable) populations did show greater fruit size than natural (less variable) populations (Blais and Lechowicz 1989). Moran et al. (1981) also found significant genetic variability and phenotypic plasticity between four colonizing races of the same annual in Australia. Although they suggested that the latter was the major mode of adaptation in this species, they concluded colonizing success was based on fecundity differences between the races. Neither one of these studies considered agrestal populations. In this study potential for invasion does not seem to be influenced by genetically based plasticity, as there was no differential temperature selection, but laboratory studies are known to underestimate this component (Venable 1984). In less marginal areas with a lower temperature amplitude, plasticity in the germination traits of EBN would presumably allow invasion of most habitats. This "general purpose" genotype (Baker 1974) appears to diverge under extreme temperatures that would be prevalent in geographically marginal areas.

Intrapopulation variation was evident in almost all populations, for germination extent, rate and seed size. A number of other studies have also observed significant genetic differences among individuals in germination profiles (Cavers and Harper 1966; Jain 1982; Wu et al. 1987; Wulff 1988; Lush 1989). Such variation in germination profiles may result in differential establishment, with the potential for variable invasion success among individuals within a population (Wulff 1988). Significant genetic effects may also be confounded by differences in phenotypic maternal effects and differences in amount of outcrossing (Shaw 1986). In the present study, these maternal effects have been minimized by raising the parentals in common conditions, followed by self-pollination.

Temperature is the main factor influencing emergence times of many weeds in mesic temperate areas (Baskin and Baskin 1988) but these patterns are modified by other factors such as soil moisture (Weaver et al. 1988), nitrogen availability (Karssen 1980/81), disturbance rates (Bazzaz 1983) and interactions among these factors. Genetic differences documented in controlled environments may be overwhelmed by phenotypic responses to environmental variation common in the field (Venable 1984). Unfortunately long term field studies to test if inter- and intra-population differences in germination profiles occur in nature, and hence control invasion potential, are complicated by the light requirement for germination.

Several observations suggest that differences observed in the lab are also present in nature. In Ontario, peak germination of northern agrestals occurs after spring planting from mid-May to early June (Weaver, unpub. data) while casual observation of field emergence indicates peak germination in the ruderal population occurs earlier in May (Hermanutz, pers. obser.). Also, the base temperature generated for the Harrow population in this study match a soil based estimate obtained by Weaver et al. (1988), sampling in the same locality. Bazzaz (1984) successfully linked ability to germinate at low temperature (from laboratory studies) to early field emergence in the annual weed Ambrosia trifida.

Population differentiation in rate of germination of EBN may be attributable to differential selection factors in ruderal and agrestal habitats, but field measurements of selection coefficients must be attempted to confirm this finding. The role of plasticity in colonization potential may be limited in marginal localities of S. ptycanthum where extreme temperatures are more prevalent. The high levels of genetic variation suggest there is ample potential for future range expansion.

Chapter 4--HERBICIDE TOLERANCE

4.1 Introduction

Once a weed invades an agrestal habitat, the most obvious selection pressure encountered is the farmer's determination to eradicate it (Barrett 1988). With the introduction of chemical herbicides some 40 years ago, traditional methods of weed control such as hoeing, cultivation and crop rotation were almost abandoned in favour of chemical control (Haas and Streibig 1982). Intensive, repeated useage of herbicides has established the potential for the evolution of herbicide tolerance or even resistance (LeBaron and Gressel 1982, pg. xv). Such a radical change in farming practise modified both the species composition, and structure of weed communities associated with most crops (Haas and Streibig 1982).

Within a species the development of herbicide tolerance or resistance is dependent on the amount of genetic variation within invading populations and its mode of inheritance (Warwick 1991), and upon the selection intensities associated with the various cultivation and rotation regimes (Holliday and Putwain 1980; Stephenson, Dykstra, McLaren and Hamill 1990). Factors such as germination dynamics, other life history traits (e.g., generation time; seed reserve and carryover), the mixture of herbicides applied and their effective kill rates will also determine the rate of appearance of tolerance or resistance

(Maxwell, Roush and Radosevich 1990; Warwick 1991).

Price et al. (1983) found that populations of Avena ssp. (wild oats) previously unexposed to herbicides harboured significant inter- and intra-population variability in tolerance, suggesting successful colonization of agrestal habitats by ruderal populations would be possible. Other studies (Jana and Naylor 1982; Holliday and Putwain 1980; Thai, Jana and Naylor 1985) have detected increased tolerance with recurrent exposure to a variety of herbicides. Increased tolerance of exposed agrestal populations could preempt colonization of agroecosystems by ruderal populations. The predominantly autogamous nature of EBN (Chapter 2) would serve to rapidly replicate these tolerant genotypes.

The difference between resistance and tolerance is one of degree: tolerance refers to the normal variability found within a species, with some individuals showing a decreased response to low herbicide doseages, while resistance is defined as a lack of response to a herbicide at normal field doses in a species which is normally susceptible (LeBaron and Gressel 1982, Warwick 1991). Variability in tolerance may either be a result of differences in herbicide uptake and translocation, or via differences in plant metabolism and herbicide detoxification (Bandeen et al. 1982; Warwick 1991). Data on tolerance levels within weed populations is scarce, especially in non-agrestal populations (Warwick

1991). In contrast resistance is conferred via alteration of site of action at the cellular level, such that individuals are resistant (R-biotype) or susceptible (S-biotype)(Warwick 1991). Recent surveys of the occurrence of resistant weed species show that the most widespread or common type of resistance is to the symmetrical-triazines (atrazine) (Gressel et al. 1982; Holt and LeBaron 1990; Warwick 1991). Although atrazine resistant populations of black nightshade (S. nigrum) have been reported in Europe since the mid-1970s (Gressel et al. 1982), no such resistant populations have been noted for EBN (Jacobs, Duesing, Antonvics and Patterson 1988, Warwick 1991).

Since its introduction in the early 1970s, metribuzin (4-amino-6-tert-butyl-3-(methylthio)-as-triazin-5(4H)-one) has been widely used by growers to control annual broadleaf species (e.g., cocklebur, lamb's quarters, pigweed and velvetleaf) and grasses in processing tomatoes. Metribuzin is an asymmetric triazine. Resistance to this herbicide has not been encountered in any weed (Bandeen et al. 1982; Warwick 1991), but cultivars of several crops (e.g. tomato, potato, soybean and wheat) do show tolerance via differential metabolism (Radosevich and Holt 1984). Therefore, differences in the degree of response of EBN to metribuzin application would likely be due to tolerance rather than resistance.

In transplanted tomatoes, metribuzin is usually applied

either as a preplant incorporated herbicide in combination with trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) or as a postemergence herbicide (OMAF 1989). Metribuzin was not specifically developed for the control of EBN, and most growers have not achieved adequate control of it with this herbicide regime (Weaver et al. 1987). Annual nightshade species, especially hairy nightshade and EBN, are difficult to control in solanaceous crops (such as tomatoes) because of their similar growth habit and physiology, and as a result often cause significant yield reductions (Weaver et al. 1987). Although EBN is more tolerant of metribuzin than many other broad-leaved weeds, populations vary in susceptibility (Ogg 1986). Degree of control of EBN is quite variable depending on year, locality and treatment history. Much of this variability would appear to be due to differences in environment, but observed differences in herbicide efficacy may be genetically based, i.e., population based differences in tolerance.

Each population in the present study possesses a different history of herbicide treatment. Both northern agrestal populations had been exposed to metribuzin for at least five years. The Harrow field had been treated with metribuzin for 8-10 years while planted in transplant tomatoes. Prior to the tomato crops, an established orchard was sprayed with either simazine or atrazine. The Wright field had grown transplanted tomatoes rotated with corn (2

year rotation) since 1979, so exposure to metribuzin would have been for 5 growing seasons at the time of collection. The corn crops had been treated with a combination of butylate* (Sutan*) and metolachlor/atrazine (Primextra). Metribuzin is not used in the southern transplant farms due to damage caused to young tomato seedlings. Instead napropamide (2-(α-naphthoxy)-N,N-diethylpropionamide) (Devrinol) is used for chemical weed control, along with hoeing. Control with this herbicide is via inhibition of root growth of germinating seeds. Napropamide controls many of the common weeds, but does not control nightshade (OMAF 1989). In Georgia, tomato transplants have been grown for at least 20 years, while tomatoes have been grown in North Carolina for only 5 years. In North Carolina, tobacco was grown prior to tomatoes. The northern ruderal populations are presumed to have never been exposed to any type of herbicide.

To test genetic and plastic differences in herbicide tolerance among and within populations, I compared the growth response of seedlings of northern ruderal, northern agrestal and southern agrestal populations to varying levels of metribuzin.

4.2 Materials and methods

To ensure uniform emergence, seeds were imbibed for 2 days under optimal conditions of 30/20°C (14 hr light during

elevated temperature). Light levels were approximately 30 umolesm^{-2}s^{1} (PAR) during imbibition in the incubator. The conditioned seeds were then planted into flats containing ABS® planting mixture, covered with vermiculite, and placed in a 28/20°C greenhouse. Additional illumination was provided by 400 W high pressure sodium lamps. Seedlings began to emerge the following day and some individuals from all families had emerged within 24 hours. Twelve days later, 16 even sized seedlings/family were transplanted individually into 9 cm plastic pots filled with 500 ml of soil (25% peat: 75% sterilized field soil). Peat was added to the soil mixture to avoid compaction, as the field soil (Field Q, Harrow Research Station) is predominately a fine grained Fox sandy loam (82.5% sand: 5% silt: 12.5% clay). It was felt that the ratio of peat:soil would not alter herbicide uptake (J. Gaynor, pers. comm.). At transplantation, and weekly thereafter, the seedlings were fertilized with 100 ml 0.9 g/l 20-20-20 NPK (Peters® Professional Mix). The pots were randomly placed on greenhouse benches and rotated every second day. Greenhouse conditions were kept as above.

Ten days after transplanting when plants were at the 5-6 leaf stage, each plant was measured (height, number of leaves, length and width of 3rd leaf) and randomly assigned to a treatment group: Control (water only--3 seedlings); medium (0.4 kg ai (active ingredient)/ha--5 seedlings) and

high (0.9 kg ai/ha--8 seedlings) levels of metribuzin. The recommended postemergence application rate of metribuzin for transplanted tomatoes is 0.25-0.85 kg ai/ha (OMAF 1989). Because of control problems and the lack of a registered alternative, growers tend to apply the upper range of the recommended dose. A commercial formulation of metribuzin (Lexone 75 DF) was used. The herbicide was applied with a Teejet 8002-E (Spraying Systems Co.) flat fan nozzle within a spray chamber (1.49 m^2) at a volume of 288 L water/ha (regulated with CO_2). The nozzle was held 45 cm above the plants. This system ensures 100% herbicide coverage with no overlap, or change in concentration. Plants were sprayed with the appropriate concentration of metribuzin within four hours of measurement, and randomly replaced on greenhouse benches. Greenhouse temperatures were 23/18°C ± 3°C, and natural light was supplemented by high pressure sodium lights to provide a 14 hr photoperiod. Plants were carefully watered from the base to avoid contact with leaf surface. Plants were remeasured 5 days post-herbicide application.

Interpopulation variability in leaf mortality (i.e., leaf completely dried out) at each herbicide level was tested using the G-test (Sokal and Rohlf 1981). The suppression of juvenile growth in height and laminar expansion rate (growth in length and width over 5 days) of the 3rd leaf at each treatment level (medium or high) was

visually compared to the control via norm of reaction plots, but the lines joining the symbols do not imply knowledge of intermediate nutested herbicide rates. Genetic variability and plasticity (environmental interactions) among and within populations (and types) were contrasted over the herbicide gradient. The amount of plasticity among populations in the growth rate characters was compared. In the case of leaf mortality, laminar expansion was designated as zero (i.e., no growth). Growth rates (height, leaf length and width) were square root transformed to meet the assumptions of ANOVA. Methods of statistical analysis are described in section 3.2.3.

4.3 Results and discussion

4.3.1 Variation among populations

Triazines are potent photosynthetic inhibitors that are activated by light, causing chlorosis and desiccation of green tissues (Ross and Lembi 1985). This response was observed in those plants in which the 3rd leaf died. There was no leaf mortality in the control group, but populations varied in their response to both levels of herbicide (trt=0.4 kg/ha: $G_{adj.}$=33.75; trt=0.9 kg/ha: $G_{adj.}$=35.05; df=6, $p<0.05$; Table 4.1). At the medium dose level, the northern populations suffered little mortality, as did the NC families. The GA populations were sensitive to this dose level, although there was a range of response among families

Table 4.1 Leaf mortality (3rd leaf) of families associated with herbicide treatment level (0.4 and 0.9 kg/ha metribuzin) for each population. Sample sizes are 5 and 8 plants per family for each treatments, respectively

TYPE	Pop.	Family	TRT	Leaf Mortality (#, %) 0.4	0.9
NR	R	1		0 (0.0)	7 (87.5)
		2		0 (0.0)	6 (75.0)
		3		1 (20.0)	8 (100.0)
		4		0 (0.0)	7 (87.5)
		5		1 (20.0)	6 (75.0)
	P	1		1 (20.0)	3 (37.5)
		2		0 (0.0)	3 (37.5)
		3		1 (0.0)	6 (75.0)
		4		1 (0.0)	2 (25.0)
		5		1 (20.0)	6 (75.0)
NA	H	1		1 (0.0)	0 (0.0)
		2		0 (0.0)	1 (12.5)
		3		1 (0.0)	1 (12.5)
		4		1 (0.0)	2 (25.0)
		5		1 (20.0)	3 (37.5)
	W	1		0 (0.0)	3 (37.5)
		2		1 (20.0)	5 (62.5)
		3		0 (0.0)	0 (0.0)
		4		0 (0.0)	0 (0.0)
		5		0 (0.0)	0 (0.0)
SA	G1	1		5 (100.0)	8 (100.0)
		2		3 (60.0)	8 (100.0)
		3		1 (20.0)	8 (100.0)
	G2	1		4 (80.0)	8 (100.0)
		2		5 (100.0)	8 (100.0)
		3		5 (100.0)	8 (100.0)
		4		4 (80.0)	8 (100.0)
		5		4 (80.0)	8 (100.0)
	NC	1		0 (0.0)	1 (12.5)
		2		0 (0.0)	3 (37.5)
		3		1 (20.0)	2 (52.0)
		4		1 (20.0)	6 (75.0)
		5		0 (0.0)	2 (25.0)

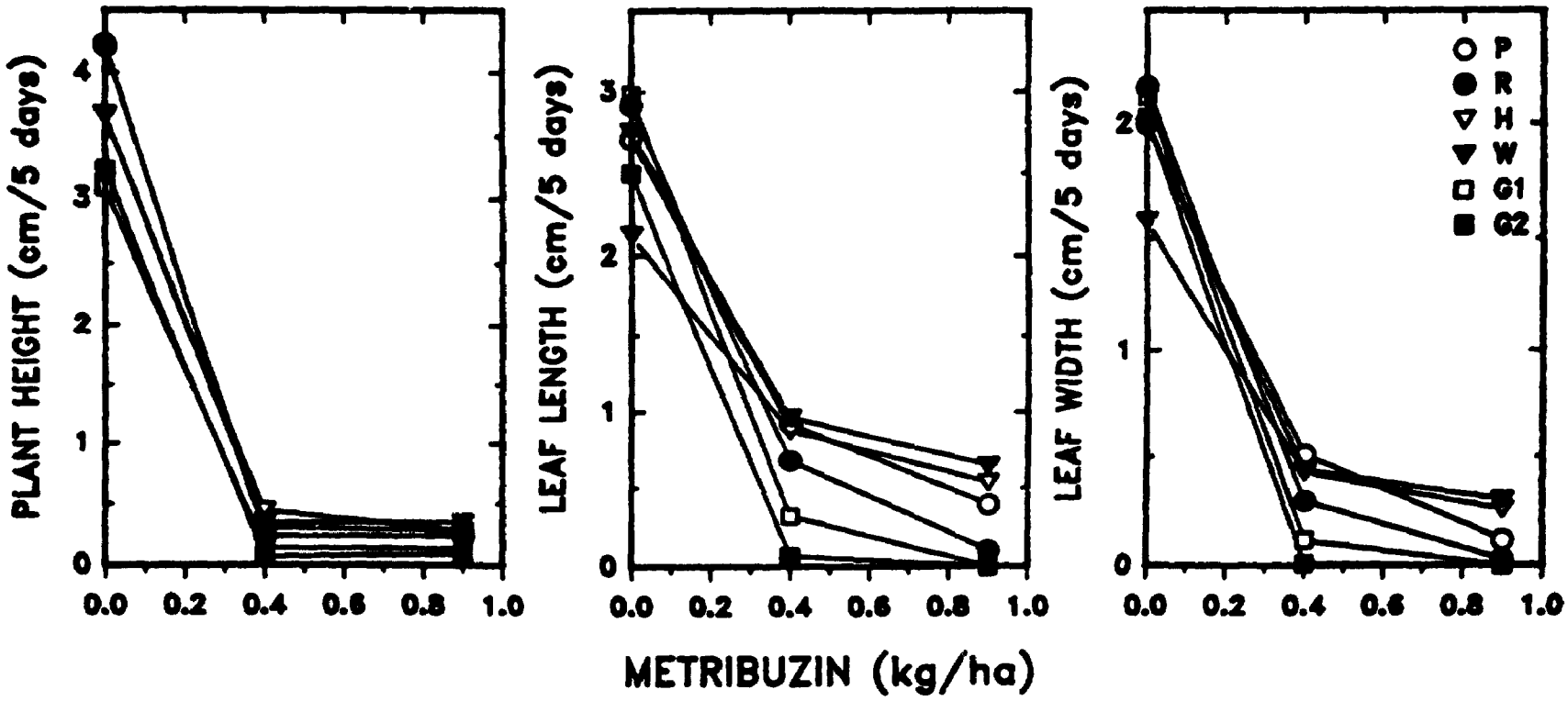
in the G1 population. The medium dose level did not discriminate between northern types, but the high dose certainly did: NR populations were more sensitive than the NA populations. Variability in leaf mortality was greatest within the P, W and NC populations. The GA populations were the most susceptible (3rd leaves of all individuals died at the highest concentration).

At first measure (22 days after sowing), populations varied in size (Table 4.2). There was significant among-population differentiation in all pre-application size characters (i.e., height, no. of leaves, length and width of the 3rd leaf: Kruskal-Wallis $x^2>69.9$, $p<0.0001$). Inter-population variation in juvenile characters is common in other weeds with a similar historical background (Warwick 1990b), and an autogamous breeding system. The Rondeau population had the largest leaves, while the NC population had the smallest. Pelee was the tallest in height, and NC was the shortest. Differences in the growth rates (in the control treatment) were also evident (Table 4.2; Fig. 4.1). Ruderal populations (R and P) had significantly greater height growth rates than northern agrestals ($F_{[1,22]}=6.11$; $p<0.02$), and NA populations grew faster than SA populations (excluding NC-$F_{[1,22]}=5.73$; $p<0.03$). Plants within the Harrow population grew the least in leaf length ($F_{[1,22]}=5.55$; $p<0.03$). The NC population had leaf growth rates similar to the Harrow population, but had suppressed height growth

Table 4.2 Population size variability (mean ± SD) of seedlings at 22 days from sowing, and growth rates (over 5 days) of untreated (control) group

Character	TYPE/Pop NR		NA		SA		
	R	P	H	W	G1	G2	NC
Height (cm) (22 days)	3.7 (0.30)	4.5 (0.81)	3.6 (0.29)	3.7 (0.31)	3.2 (0.33)	3.4 (0.34)	2.8 (0.35)
Height increase (cm;22-27 days)	4.2 (0.70)	4.2 (0.72)	3.7 (0.59)	3.7 (0.34)	3.1 (0.71)	3.2 (0.81)	2.8 (0.31)
Leaf number	5.7 (0.62)	5.8 (0.50)	6.4 (0.55)	6.2 (0.46)	5.1 (0.36)	5.3 (0.49)	5.6 (0.50)
Leaf length (cm)	5.2 (0.49)	5.0 (0.74)	4.6 (0.48)	4.8 (0.42)	5.2 (0.54)	5.1 (0.50)	4.4 (0.42)
Length increase (cm)	2.7 (0.42)	2.9 (0.54)	2.1 (0.73)	2.8 (0.63)	3.0 (0.46)	2.5 (0.67)	2.0 (0.39)
Leaf width (cm)	3.6 (0.35)	3.4 (0.45)	3.3 (0.35)	3.4 (0.30)	3.5 (0.50)	3.6 (0.45)	3.2 (0.30)
Width increase (cm)	2.0 (0.36)	2.2 (0.32)	1.6 (0.61)	2.0 (0.44)	2.1 (0.45)	2.0 (0.48)	1.5 (0.33)

Fig. 4.1 Population response curves of EBN for growth in height, leaf length and width (of 3rd leaf) as a function of metribuzin concentration. Note NC is included in this figure but not in the ANOVA (Table 4.3). Northern Ruderal- P, R; Northern Agrestal- H, W and Southern Agrestal- G1, G2, NC.


PLANT HEIGHT (cm/5 days)
LEAF LENGTH (cm/5 days)
LEAF WIDTH (cm/5 days)
METRIBUZIN (kg/ha)
0.0
0.2
0.4
0.6
0.8
1.0
0
1
2
3
4
P
R
H
W
G1
G2

(Table 4.2; Fig. 4.1). Northern agrestal populations did not appear to have increased juvenile growth rates compared to ruderal populations under greenhouse conditions.

There were significant differences among populations in the growth rate responses to metribuzin for all characters (Table 4.3). There was no size bias associated with the initial allocation to each treatment level (Appendix D). Nor was the variability in growth response a consequence of the pre-application size differences among populations (Appendix D). Although it is difficult to detect height differences in Fig. 4.1 because of the relatively complete overall suppression of growth, both northern types (agrestal and ruderal) were significantly more tolerant of both concentrations of metribuzin than the southern populations (NA vs NR: all $F_{[1,22]}<3.66$; $p>0.05$, and NA vs SA: all $F_{[1,22]}>12.27$; $p<0.0002$). Changes in leaf size (length and width) revealed greater variability among populations to the herbicide, with the NA type significantly more tolerant than the SA type at both dose levels (all $F_{[1,22]}>20.4$; $p<0.001$, Fig. 4.1). At the medium dose level, similar leaf length and width growth rates indicated the NR type was as tolerant as the NA type ($F_{[1,22]}<1.14$; $p>0.05$).

As with leaf mortality, the highest dose differentiates the northern agrestal and ruderal populations in the amount of leaf growth ($F_{[1,22]}>12.20$; $p<0.002$). As was the case with germination response, populations within the NR type vary in

Table 4.3 Mixed model ANOVA of height, leaf length and width growth among populations (excluding NC) in response to herbicide treatment (Trt). All F ratios are significant at P < 0.001 unless otherwise noted

		Height		Length		Width	
SOURCE	df	MS	F	MS	F	MS	F
Trt	2	759.08	593.57	401.02	59.69	343.80	99.39
Population	5	10.55	16.58	17.56	30.24	5.24	14.11
T X P	10	1.28	2.01*	6.72	11.57	3.46	9.31
Error	430	0.64		0.58		0.37	

* P < 0.05

their response at the higher herbicide level, with Pelee more tolerant than Rondeau (Fig. 4.1). When the NC population was included in the analysis, it exhibited a much higher tolerance in leaf growth rate compared to the other southern populations, ($F_{[1,22]}>9.72$; $p<0.005$).

This experiment was carried out in the greenhouse controlling only a single factor, rather than under more selectively stringent conditions found in the field. Additional selection pressures generated by competitive interactions with crops and other weed species, cultivation regimes, fertilizer applications and their synergistic interactions could change the selective environment. Variability in other life history characters, such as the delayed germination found in the NA and SA types compared with the earlier germinating NR type (see Chapter 3) may prevent or retard the evolution of more tolerant populations. In contrast, Putwain, Scott and Holliday (1982) found that a switch from a summer to a winter annual life cycle by a herbicide treated population of Senecio vulgaris was not the consequence of delayed germination (lack of heritable variation), but rather due to the fact that available germination microsites occurred only in the fall in the herbicide treated site. The role of the seed bank in buffering the selective force of herbicides must also be considered (Gressel and Segel 1978; Maxell et al. 1990). Although the buried seed population is small in

comparison to the annual seed input (Weaver and Hermanutz, unpub. data), the majority of seeds are incorporated back into the seed pool, and constant cultivation allows recruitment from the seed bank. Extremely susceptible individuals within the agrestal populations may have been generated by residual dormant seeds produced during pre-metribuzin treatment times. Such dormancy mechanisms slow response to selection (Jana and Thai 1987). If inheritance for tolerance to metribuzin is determined in the nuclear genome and multigenic (inheritance pattern is not known), restricted recombination due to the autogamous breeding system would also slow evolution (Jana and Naylor 1982).

All populations showed high levels of plasticity for all growth rate characters, showing a "tolerant" rather than "resistant" nature (Table 4.3). Population X treatment interactions were significant for all juvenile growth characters. Cross-overs occurred between the control and "low" herbicide doses among northern and southern populations (Fig. 4.1) indicating potential selection against nightshade originating from Georgia in agroecosystems using this herbicide, and thus limited invasive potential in this particular system. Although it is difficult to compare the response to greenhouse rates with those obtained in the field because of increased herbicide efficacy under greenhouse conditions, it seems that NR populations could potentially invade agroecosystems.

At the higher herbicide concentration suppression in growth was greater in the NR populations, and this would select NA populations. Nevertheless, it appears that individuals from the NC population could potentially invade and survive within the northern agrestal habitat. Warwick and Marriage (1982) found no evidence for the potential for herbicide-dependent selection (i.e., absence of population X treatment interaction) among susceptible populations of Chenopodium album, but populations did differ in most seedling characters. The higher tolerance of the northern agrestal populations was expressed as a decreased amount of plasticity for all characters when compared to the other types (Table 4.4). The NC population falls within the bounds of the northern agrestal populations.

4.3.2 Variation within populations

With few exceptions, genetic variability within individual populations was low (Table 4.5; Fig. 4.2-4.5), with maximum variability expressed in the control treatment for all growth characters (Table 4.6). Increased efficacy of the herbicide in the greenhouse may have masked the extent of variability found within these populations. The northern agrestal populations showed significant between-family variability in height (W) or leaf (H) growth rates (Table 4.5), suggesting that herbicide useage has not selectively removed less tolerant families. Mechanisms which buffer the impact of herbicides could result in the maintenance of

Table 4.4 Amount of plasticity in height, third leaf length and width growth rate across a herbicide gradient, measured by the CV (SE) for each population of EBN. Probability values based on Kruskal-Wallis test for type and population differentiation

TYPE/ Population	Coefficient of Variation (SE)		
	Height	Length	Width
NORTHERN RUDERAL			
Pelee	158.6 (6.14)	98.7 (8.97)	129.0 (8.00)
Rondeau	168.0 (7.45)	138.9 (6.54)	166.7 (6.36)
NORTHERN AGRESTAL			
Harrow	143.6 (7.99)	77.1 (10.91)	113.3 (17.61)
Wright	142.6 (10.75)	81.0 (13.64)	111.4 (18.67)
SOUTHERN AGRESTAL			
G2	192.4 (7.11)	208.4 (4.73)	216.2 (1.58)
G1	175.8 (13.48)	184.7 (16.46)	200.0 (7.98)
NC	174.4 (8.82)	95.3 (12.34)	109.1 (11.72)
TYPE[a]	p=0.0021	p=0.0001	p=0.0001
POPULATION	p=0.0183	p=0.0004	p=0.0007

[a] NC population is not included in this test.

Table 4.5 Mixed model ANOVA of each growth rate trait (height (A), length (B) and width (C) of the 3rd leaf) for each population of EBN in response to herbicide treatment (Trt). Mean square values and the significance of the F ratio are shown (df=2,4,8,65 except for G1 df=2,2,4,39)

SOURCE	TYPE/POPULATION						
	NR		NA		SA		
	Pelee	Rondeau	Harrow	Wright	G2	G1	NC
A.							
Trt[a]	150.3	165.4	121.9	118.7	159.5	80.5	124.7
Family	0.6ns	2.4*	0.8ns	2.0*	1.0ns	1.4ns	1.2ns
T X F	0.8ns	0.2ns	1.0ns	0.6ns	0.6ns	1.1ns	0.3ns
Error	0.5	0.6	0.6	0.5	0.5	0.5	0.7
B.							
Trt[a]	66.2	108.0	29.7	43.3	108.8	75.8	39.0
Family	0.4ns	0.8ns	4.8[a]	1.0ns	0.7ns	1.4*	0.6ns
T X F	0.8ns	0.2ns	0.4ns	1.2*	0.5ns	1.1*	0.5ns
Error	0.9	0.5	0.5	0.5	0.2	0.3	0.6
C.							
Trt[a]	64.1	81.9	32.5	45.1	88.4	53.0	34.8
Family	0.0ns	0.2ns	5.2[a]	0.5ns	0.4*	0.4ns	0.4ns
T X F	0.3ns	0.2ns	0.3ns	1.1*	0.2*	0.2ns	0.6ns
Error	0.5	0.3	0.3	0.4	0.0	0.2	0.4

[a] $P < 0.0001$; * $P < 0.01$; ns=non-significant

Fig. 4.2 Norm of reaction plots of each population (excluding NC; see Fig. 4.3) of EBN to show variation among five (or three for G1) families for growth in height as a function of metribuzin concentration. Symbols denote individual families within a population.

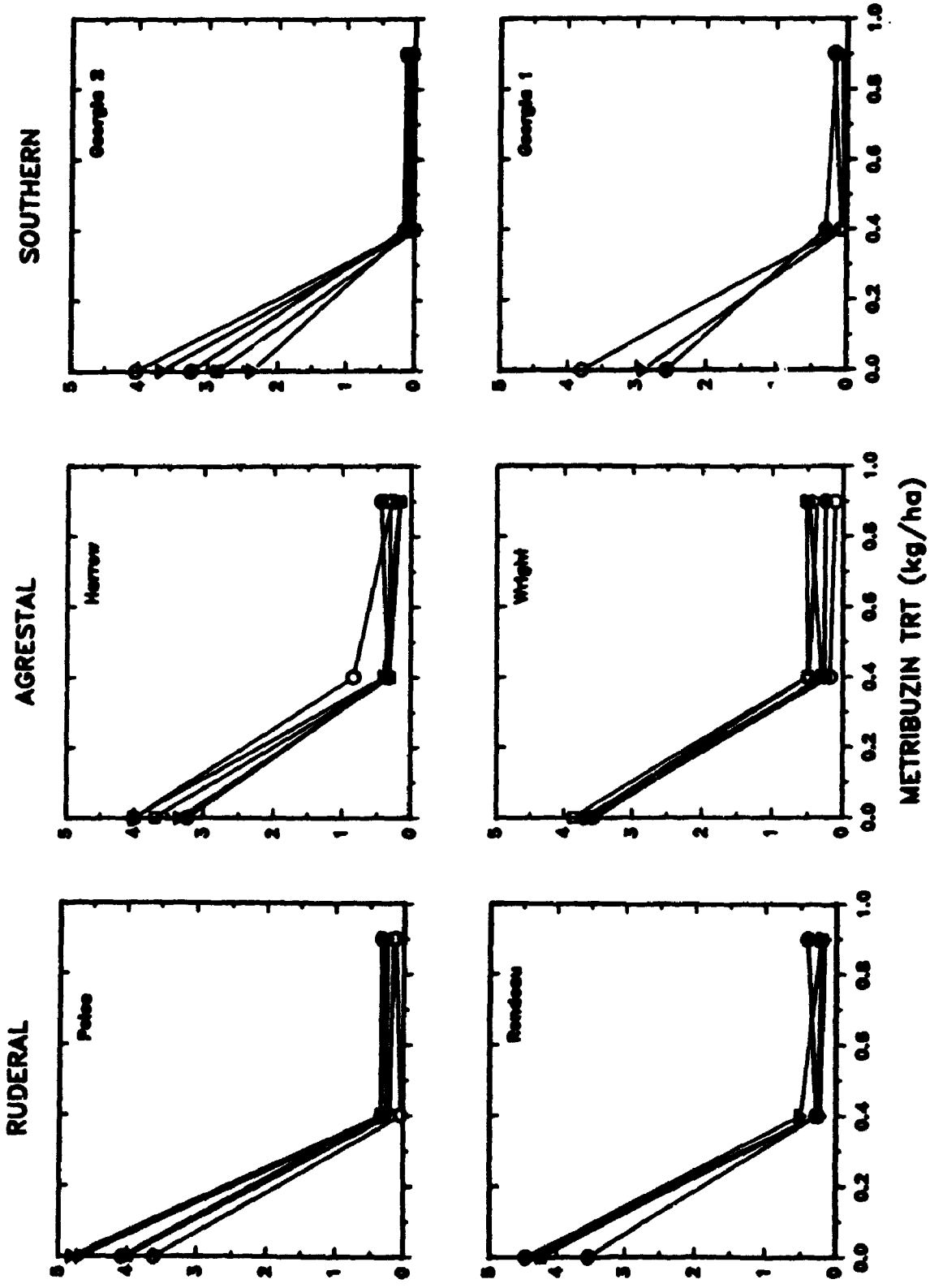

SOUTHERN
AGRESTAL
RUDERAL
Georgia 2
Georgia 1
Harrow
Wright
Pelee
Rondeau
HEIGHT GROWTH (cm/5 days)
METRIBUZIN TRT (kg/ha)

Fig. 4.3 Norm of reaction plots of the North Carolina population showing the five families for each of the three growth characters as a function of metribuzin concentration.

NORTH CAROLINA

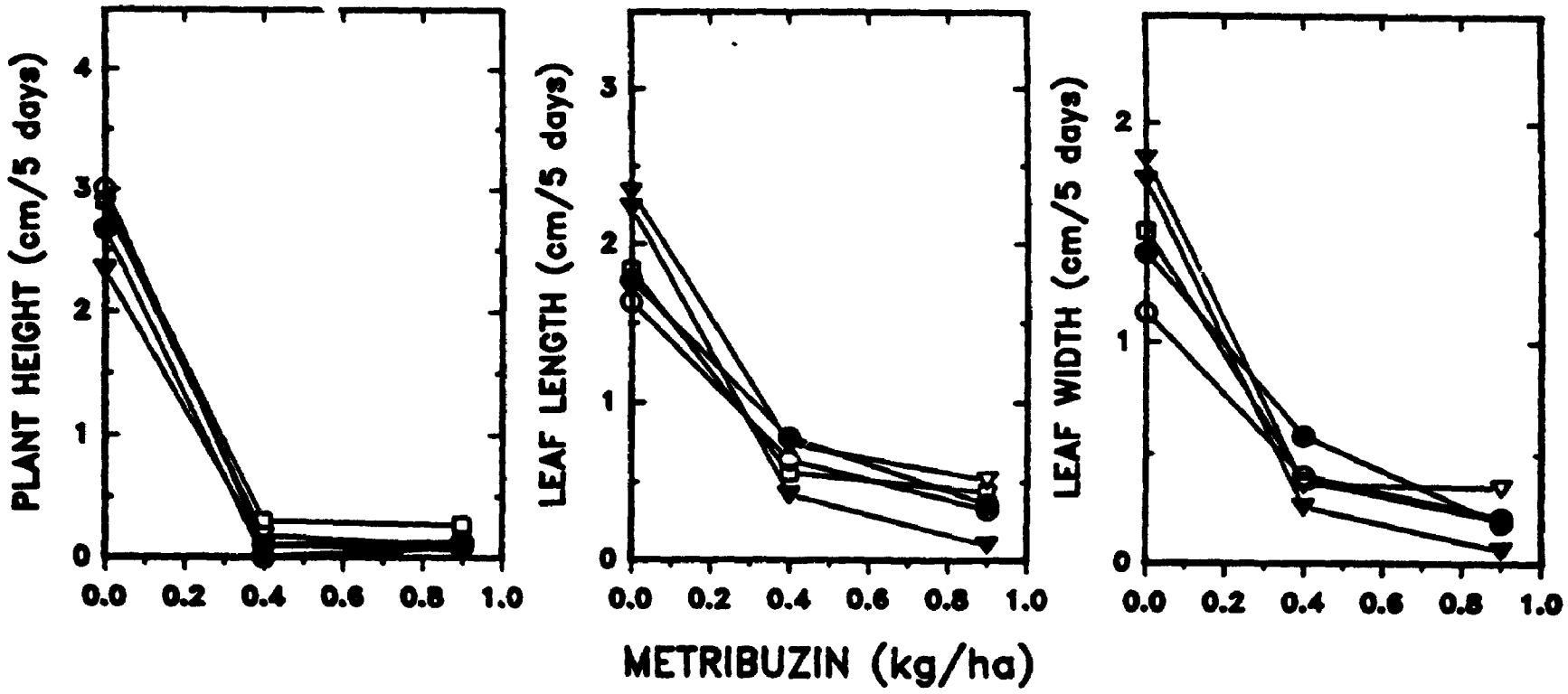


Fig. 4.4 Norm of reaction plots of each population to show variation among families for growth in length of the 3rd leaf as a function of metribuzin concentration.

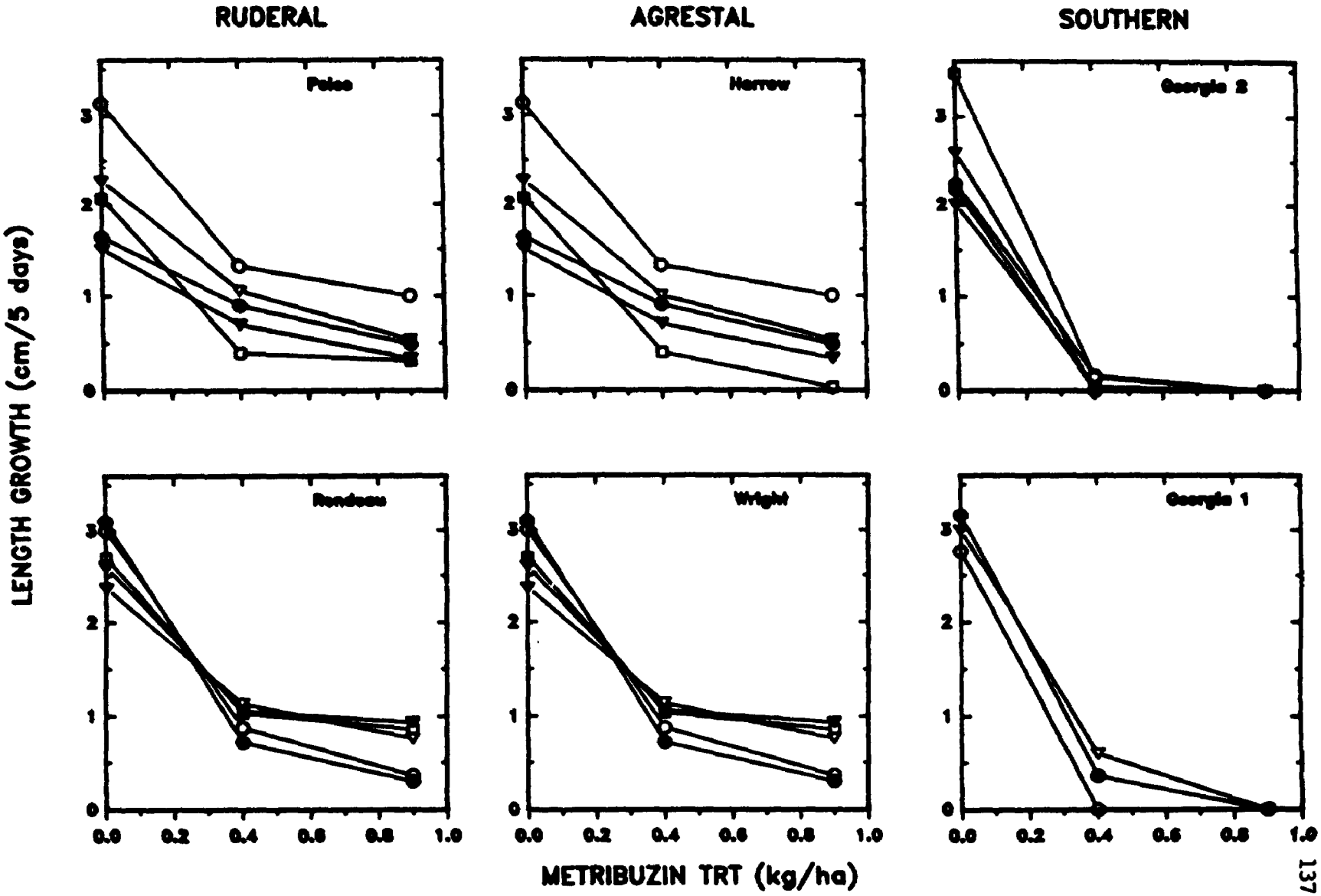

RUDERAL
AGRESTAL
SOUTHERN
Palco
Narrow
Georgia 2
Rondeau
Wright
Georgia 1
LENGTH GROWTH (cm/5 days)
METRIBUZIN TRT (kg/ha)
0
1
2
3
0.0
0.2
0.4
0.6
0.8
1.0

Fig. 4.5 Norm of reaction plots of each population to show variation among families for growth in width of the 3rd leaf as a function of metribuzin concentration.

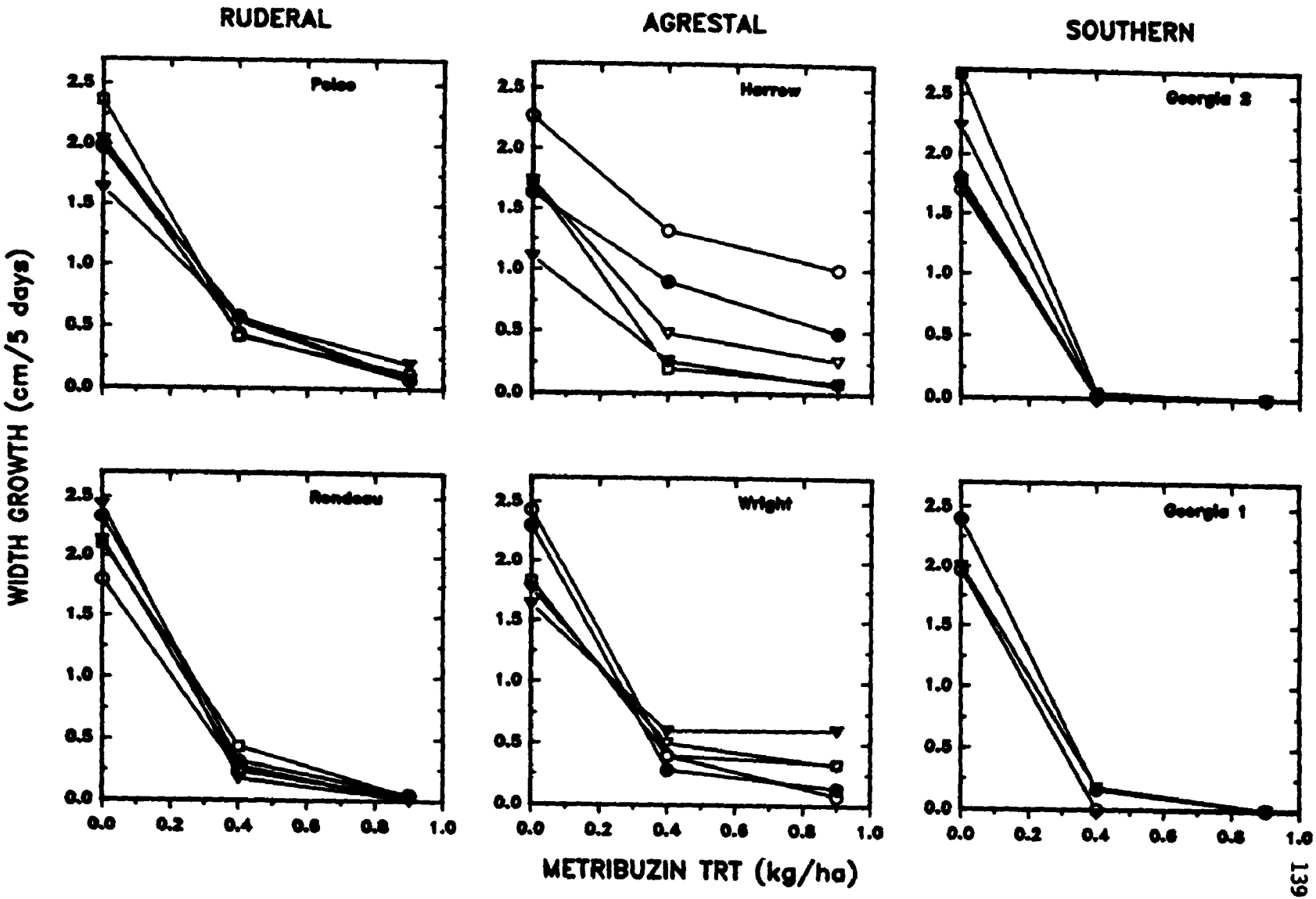

RUDERAL
AGRESTAL
SOUTHERN
Peloe
Harrow
Georgia 2
Rondeau
Wright
Georgia 1
WIDTH GROWTH (cm/5 days)
METRIBUZIN TRT (kg/ha)
0.0
0.5
1.0
1.5
2.0
2.5
0.2
0.4
0.6
0.8

Table 4.6 Percentage variance accounted for, and the significance of the F ratios among populations (excluding NC) of EBN, and among families nested within population, for each herbicide treatment level for growth in height (A), length (B) and width of the 3rd leaf (All F values significant at P < 0.001, unless otherwise noted; df=5,22)

SOURCE OF VARIATION	METRIBUZIN TRT (kg/ha) 0.0	0.4	0.9
A.			
Population	26.81	28.99	12.42*
Family(Pop)	17.49	10.61	15.77
R^2	0.61	0.49	0.35
B.			
Population	15.26ns	54.04	45.98
Family(Pop)	29.82	7.96*	11.06
R^2	0.62	0.66	0.59
C.			
Population	10.89ns	42.41	26.42*
Family(Pop)	35.88	6.18ns	24.95
R^2	0.63	0.55	0.55

* P < 0.05; ns=non-significant

variable populations (see above section). Significant levels of intrapopulation variation in a few of the populations (assuming a portion is additive genetic variance), suggest that ability to respond to selection may differ among populations.

Within populations, the lack of significant levels of plasticity (i.e., family X treatment) in different families suggests the role of herbicide-dependent selection would be minimal. Within the Wright population several families are equally tolerant to both herbicide doses (Table 4.5), compared with the others which are negatively affected, generating significant interactions (direction rather than cross-overs). In contrast to results for temperature-dependent germination characters, there appears to be much less intrapopulation variability in herbicide tolerance, with the majority of the variation apportioned among populations, i.e. populations means have diverged (Table 3.5, 4.6). This may be a result of the limited number of families tested.

With the exception of triazine resistance, few studies have investigated the amount and/or distribution of genetic variation among and within populations in response to herbicides (Warwick 1990a). Price et al. (1983) documented significant intra-population variability for tolerance in 2 of 4 populations of wild oats, all of which were previously unexposed to herbicide. Holliday and Putwain (1980) found

significant among family variability in the S_2 generation of Senecio vulgaris.

When applied to the foliage, photosynthetic inhibitors act as contact herbicides, and if the seedling survives, reproductive output is not affected (da Silva and Warren 1976). Regardless of the amount of suppression of juvenile growth in response to herbicide, all individuals did successfully produce flowers (dates not recorded and plants were discarded before all set berries). Hume and Shirriff (1989) have shown that plants (Chenopodium album) treated with 2,4-D were smaller, took longer to initiate flowering, and produced fewer, but larger seeds which also germinated faster than those from control plants. They suggested that these seed characters may confer a competitive advantage to sprayed plants. In their study however, populations with varying tolerance levels showed little variation in their reproductive characters in response to herbicide. It is of interest to note that the most tolerant population in their study was a population which had had no herbicide for more than 15 years. In tomato fields, plants from the Harrow population of EBN sprayed with metribuzin often abort the first flower cluster, and produce berries with fewer, smaller seeds (K. Ward, unpub. data). Unfortunately, we know little of the reproductive dynamics of the ruderal or southern populations in response to herbicide. Decreased juvenile growth rates in response to herbicide application

in southern and ruderal populations may decrease competitive ability. An increased length of the pre-reproductive phase and/or decreased reproductive output may result in the inability of these populations to successfully invade agrestal habitats.

CHAPTER 5-VARIABILITY IN RESPONSE TO A NUTRIENT GRADIENT

5.1 Introduction

Simmonds (1981) has suggested that the "green revolution" in agriculture, with its very high inputs of nutrients has acted to select crop varieties with large positive genotype by environment interactions in yield. High yield potentials in the majority of these cultivars (e.g. rice, barley) are contingent on high fertility, with extremely poor productivity under low nutrient regimes (Simmonds 1981). Has there been concomitant selection for the ability of weeds in these agroecosystems to increase their reproductive output under highly fertile conditions, with a correspondingly low "yield" in poor habitats?

Many of the annuals associated with agriculture (e.g., Chenopodium album, Abutilion theophrasti, Amaranthus retroflexus) have the ability to rapidly increase their reproductive biomass in response to nutrients, much more than in response to other abiotic factors such as light, moisture, and temperature (Parrish and Bazzaz 1985). Within agroecosystems, competition with crops and other weeds for available nutrients (and light) may select a "competitive-ruderal" strategy (Grime 1979). Increased age of first reproduction, a decreased root:shoot ratio, decreased allocation to reproduction and larger propagule size differentiate this strategy from the non-competitive "ruderal" strategy (Anderrson 1989c).

In addition, it has been hypothesized that genetic variability within agrestal populations in response to nutrients will be less than in ruderals because of the high constant inputs of fertilizers in the agricultural habitat compared to the highly heterogeneous nutrient levels in ruderal habitats (Table 1.5; Barrett 1988). The beach environments where EBN occurs (i.e., ruderal habitats) would be expected to oscillate between high spring and fall inputs of nutrients via seasonal flooding, and summer low periods. Highly variable levels of nutrients (especially within a season) may also select for "broadly" adapted genotypes with high levels of phenotypic plasticity, allowing growth and reproduction over a wide range of resource availability (Bradshaw 1965; Lotz and Blom 1986). Given the lower nutrient inputs and unpredictability in nutrient availability associated with ruderal habitats: 1) are individuals originating from ruderal habitats capable of higher reproductive output at lower nutrient levels than those of agrestal habitats?; and 2) are there greater levels of genetic variability and/or phenotypic plasticity in response to nutrient availability in ruderal compared to agrestal populations?

To test these hypotheses, I characterized the growth and reproductive responses of populations sampled from NA and NR habitats of EBN over 3 nutrient levels in the greenhouse. To assess amount and variability of available

nitrogen in both types of northern habitats (agrestal and ruderal), long-(between seasons) and short-term (within a season) components of environmental variability were investigated by documenting the natural levels of available N over two seasons.

Several studies have shown the potential for rapid genetic change in response to increased soil fertility (see Snaydon and Davies 1982; Quinn 1987), and physiological adaptation to fertility levels (Chapin 1980; Stulen, Lanting, Lambers, Posthumus, van de Dijk and Hofstra 1981). For example, nitrogen metabolism varied with nutrient supply in a Plantago spp. originating from nutrient poor habitat, while metabolism was independent of nutrient availability in another species from a nutrient rich habitat (Stulen et al. 1981). To test differences in nitrogen uptake, residual levels of N at the termination of the experiment were determined, and then correlated to aboveground biomass (both vegetative and reproductive).

Southern populations of EBN which inhabit similar agrestal habitats are expected to respond to resource availability in a manner comparable to NA populations. Inclusion of the SA populations in this experiment allows the more recently established NA populations to be compared to populations which are older, and originate in the south area of distribution (see section 1.7.1; Chapter 3). Intraspecific comparisons along latitudinal gradients have

documented differentiation in a number of life history traits (see Warwick 1990a), such as a decrease in the pre-reproductive period from south to north (Neuffer and Hurka 1986; Potvin 1986). As cited above, nutrient availability is an appropriate factor in which to measure life history traits, as this factor shows maximal plasticity in allocation to reproduction (Parrish and Bazzaz 1985).

5.2 Materials and methods

5.2.1 Available nitrogen in agrestal and ruderal habitats

Natural nitrogen (N) levels of the Ontario sites were monitored over two years to compare the amount of available N and its seasonal variability. In 1988 two sampling dates (late July and mid-October), and in 1989 three sampling dates (mid-May, mid-August, mid-October) were chosen to coincide with germination, growth and reproduction of EBN. At each sampling date, 8-10 stations were sampled in each field (Harrow and Wright) and in each ruderal site (Pelee and Rondeau), with each station in close association with EBN plants. At each station, five to seven 10 cm deep soil cores were taken and bulked. Soil samples were kept at 4°C until extraction. Total available N was extracted following the procedure of Drury, McKenney and Findlay (1991). A 20 g (field moist) subsample of soil was weighed into a 250 ml Erlenmeyer flask and 100 ml 2N KCl added. The samples were

shaken on a rotary shaker for 1 hr and filtered through Whatman #40 filter paper. The filtrate was kept at 4°C until analyzed. The extracts were analyzed on a TRAACS 800 autoanalyzer. Ammonium (NH_4^+) content was determined using the Berthelot reaction. Nitrate (NO_3^-) plus nitrite (NO_2^-) content (ppm) was determined using a cadmium reduction method. In all cases NO_2^- content was negligible. The gravimetric moisture content of the soil samples was calculated and all results expressed on an oven dry basis (105°C). Mean NH_4^+, NO_3^- and total extractable N (sum of ammonium and nitrate), and their standard errors were calculated for each site, at each sampling time.

5.2.2 Pre-treatment plant establishment and nutrient application

S_1 seeds were planted into trays filled with a 3:1 sterilized field soil:peat mixture (Field Q, Harrow Research Station; Fox sandy loam-82.5% sand: 5% silt: 12.5% clay) mixture, covered with a plastic sheet and placed in a 28/20±2°C (day/night) greenhouse. Additional illumination was provided by 400 W high pressure sodium lamps to provide a 14 hr "day". Seeds germinated within 4-6 days, and seedlings began to emerge 2-3 days after that. To prevent fungal contamination, seedlings were watered with a benlate mixture (0.5 g/l). A week after germination, pairs of seedlings were transplanted into 6 l Polycon plastic pots filled with 5 kg (± 100 g) of sieved soil (25% peat: 75%

sterilized field soil; see above). Transplanted seedlings were approximately 12-18 mm in height with the first true leaf <5 mm in length. At transplantation, seedlings received 500 ml of assigned nutrient treatment. The pots were randomly placed on greenhouse benches and rotated every second day for the first 2 weeks, and then weekly until termination of the experiment. Greenhouse conditions were kept as described above.

Three treatment levels were chosen: 1) no additional nutrient input (H_20 only); 2) 0.91 g/l and 3) 2.72 g/l 20-20-20 N:P:K (Peters® Professional Mix). This soluble fertilizer provided phosphorus as phosphoric acid, potassium as soluble potash and chelated micro-nutrients needed for growth. Although both P and K are important additions in agricultural ecosystems, I chose to concentrate on N as EBN is a known nitrophile (Ogg and Rogers 1989). The total amount of N applied over the duration of the experiment in the above treatments was 200 mg N/kg soil (trt 2-"medium"), which corresponded to a medium field application rate (C. Drury, pers. comm.) and a "luxurious" rate (trt 3-"high"). Some N was supplied from native soil used (approximately 14 mg N/kg) and water (tap; approximately 0.112 ppm NO_3^- and below detectable levels of NH_4^+). The fertilizer was applied weekly, in a total of 5.5 l of water over the entire experiment. Plants were carefully watered each day to avoid runoff which would virtually eliminate N loss by leaching.

5.2.3 Sampling and statistical design

A single pot per family was allocated to each of the three blocks (greenhouses) in a randomized complete block design with no replication (i.e, 3 pots/family/treatment level, for a total of 297 pots; see section 1.7.1 for sampling design). Variability and plasticity of juvenile traits were documented by harvesting and measuring one of the two seedlings at 21 days of age (i.e., 2 weeks post-transplantation). Total leaf area was measured and total aboveground biomass dried and weighed. Variability in adult characters was measured on two dates: A) at the end of the pre-reproductive phase (i.e., date first flower opened) (sampled non-destructively), and B) at the termination of the experiment. All plants were harvested when the first individual ripened berries (63-65 days of age). Time of harvest was chosen to simulate an early harvest crop such as tomato. At the final harvest plants were partitioned into vegetative (leaves and stems) and reproductive (berries and flowers, plus peduncles) components, dried and weighed. Roots were recovered from the soil, washed to remove adherent soil, dried and weighed. This method of retrieval collects the majority of the fibrous root biomass. Table 5.1 lists characters measured, and the transformations applied to conform to assumptions of ANOVA.

The method of univariate analysis was similar to section 3.2.3, with the exception that the full model

Table 5.1 List of characters measured in the nutrient experiment using EBN, their abbreviations and transformations used in the ANOVA models

CHARACTER	TRANSFORMATION
A. Measured	
SEEDLING TRAITS (21 days of age):	
1) height, mm (HT1)	log
2) leaf area, mm^2 (LA)	log
3) total aboveground dry weight, g (DW)	
4) presence of flower buds (FLB)	
ADULT TRAITS:	
A) At flowering:	
5) height, cm (HT2)	log
6) height of first split, cm (SPL2)	
7) # flowers in the first cluster (FL1)	sqrt
8) # leaves below split (LV)	*
9) # branches below split (BR)	sqrt
10) # days to first flower opening in 1st cluster (AGE1)	log
B) At harvest:	
11) height, cm (HT3)	log
12) height at first split, cm (SPL3)	
13) length of 1st branch internode, cm (INTER)	log
14) length of side branch, cm (SIDE)	log
15) basal diameter, cm (BD)	log
16) angle of the split, degrees (ANG)	log
17) # berries in the 1st cluster (B1)	
18) # berries in the 2nd cluster (B2)	
19) # berries in the 3rd cluster (B3)	
20) ripeness of berries (2=ripe, 0=unripe)	
21) vegetative aboveground dry weight (VDW)	log
22) reproductive dry weight (RPDW)	log
23) root dry weight (ROOT)	
B. Derived:	
24) % fruit set of 1st cluster (BSET=B1/FL1)	arcsin sqrt
25) mean # berries / cluster (BCL=B1+B2+B3/3)	sqrt
26) reproductive:vegetative ratio (RDW=RPDW/VDW)	sqrt
27) root:shoot ratio (RTDW=ROOT/VDW)	sqrt
28) habit (HAB=SIDE/HT3)	log

* untransformed

extracting the block effect and all interactions was run initially. All interactions with the block effect were nonsignificant, and therefore only the main effects (block, treatment, population and/or family) and the environment-dependent interactions are presented. F ratios were calculated as in section 3.2.3, and block effect (random) was tested over the error (Sokal and Rohlf 1982). A priori contrasts between populations within types and among types were calculated from the nested ANOVA (see section 3.2.3). As PROC NESTED will not remove variability assigned to the block effect, the proportion of genetic variation among and within populations was recalculated from the nested ANOVA (PROC GLM) after removing the block effect from the error term. Residuals from all above models were subjected to PROC UNIVARIATE (distribution, normal probability plots and residuals plotted against predicted values) to confirm normality.

Intraclass correlation coefficients (t=among family variance component/among family + within family variance component) were calculated from the nested ANOVA (block effect removed) (Lawrence 1984; Venable and Burquez 1989). Intraclass correlations provide an estimate of the proportion of the phenotypic variance of a trait which is under genetic control (Venable 1984). The relationship of the intraclass correlation coefficient to the heritability of a trait is dependent on the breeding structure of the

population (Lawrence 1984). In fully inbred species all individuals are homozygous and within-family variance would be the result of environmental variability. Under these conditions, the intraclass correlation coefficient approximates the broad-sense heritability (Venable and Burquez 1989). Any outcrossing would increase within-family variance and decrease the t value, so that heritability could be up to twice the estimated t value (Lawrence 1984). Actual heritabilities were not calculated because the populations differed in their potentail outcrossing rates (Chapter 2), the populations were pooled, and the number of families sampled per populations was small. Intraclass coefficients were generated to obtain a relative ranking of the genetic components of the traits to one another (Lawrence 1984).

As anticipated, many of the growth measures were correlated (Appendix E), so a subset of traits was selected which represented important life history traits and were least correlated with each other (1) juvenile size: HT1, LA; 2) age and size at the onset of reproduction: AGE1, HT2, BR, INTER; and 3) measures of reproductive output or "fitness": reproductive to vegetative ratio (RDW), FL1, BCL, BSET, RPDW). Morphological traits which discriminated among populations and treatments (LV, BD, SIDE, ANG, HT3, RTDW, VDW, HABIT) were also included. Responses of these traits to the nutrient gradient are presented as norm of

reaction plots. The untransformed means pooled across blocks can be plotted as unbiased estimates because the block by treatment interactions were not significant.

Traits for which the family variance component was significant in the nested ANOVA were further analyzed for intrapopulation variability and plasticity.

To present a composite response of each type (NR, NA and SA) to nutrients, canonical discriminant analysis (Klecka 1980) using Proc CANDISC (PC-SAS 1988) was performed on family means (i.e., pooling blocks) of the following characters: AGE1, LV, HT3, VDW, RTDW, RDW, RPDW, SIDE, BR, BAS. Each of the nine habitat type-nutrient level combinations was designated as a group in the analysis (Blais and Lechowicz 1989). Distances between the group centroids were measured by the Mahalanobis distance (D^2), and significance of these distances was tested using an F ratio (Sneath and Sokal 1973; pg. 403). As the families within habitat types are replicated over each of these nutrient levels, this approach illustrates both amount of population differentiation, and overall plastic responses to resource availability of each type (Blais and Lechowicz 1989).

5.2.4 Residual N levels at the termination of the nutrient manipulation

The amount of nitrogen extracted by each population (i.e., uptake from the soil over the duration of the

experiment), was determined at the termination of the experiment. Soil was subsampled from pots of individual plants grown at all three nutrient levels using two randomly chosen families from each population. Total available nitrogen residual in the soil was analyzed following the methods described in section 5.2.3.

5.3 Results

5.3.1 Variation in available nitrogen in agrestal and ruderal habitats

Contrary to the expected high constant levels of nutrients available to plants in agroecosystems (Barrett 1988), available N varied more in northern agrestal fields (CV: H=75.80; W=70.81) than ruderal habitats (CV: P=38.70; R=35.73) over the period sampled (Fig. 5.1). Such extreme fluctuations in the nutrient regimes would suggest that plasticity, rather than differential selection based on fertility would be important in both habitats, but to a greater degree in agrestal rather than ruderal habitats. Unfortunately, fertility levels within SA habitats are not known, but based on a longer growing season, and multiple crops grown within a single season, highly variable levels would also be expected.

5.3.2 Variation among populations

Population differentiation was evident for all traits except fruit set (BSET) (Table 5.2; Fig. 5.2-5.6).

Fig. 5.1 Mean and standard error of the total available nitrogen extracted from ruderal and agrestal habitats (Pelee, Rondeau, Harrow and Wright) at sampling times:

1- July 1988

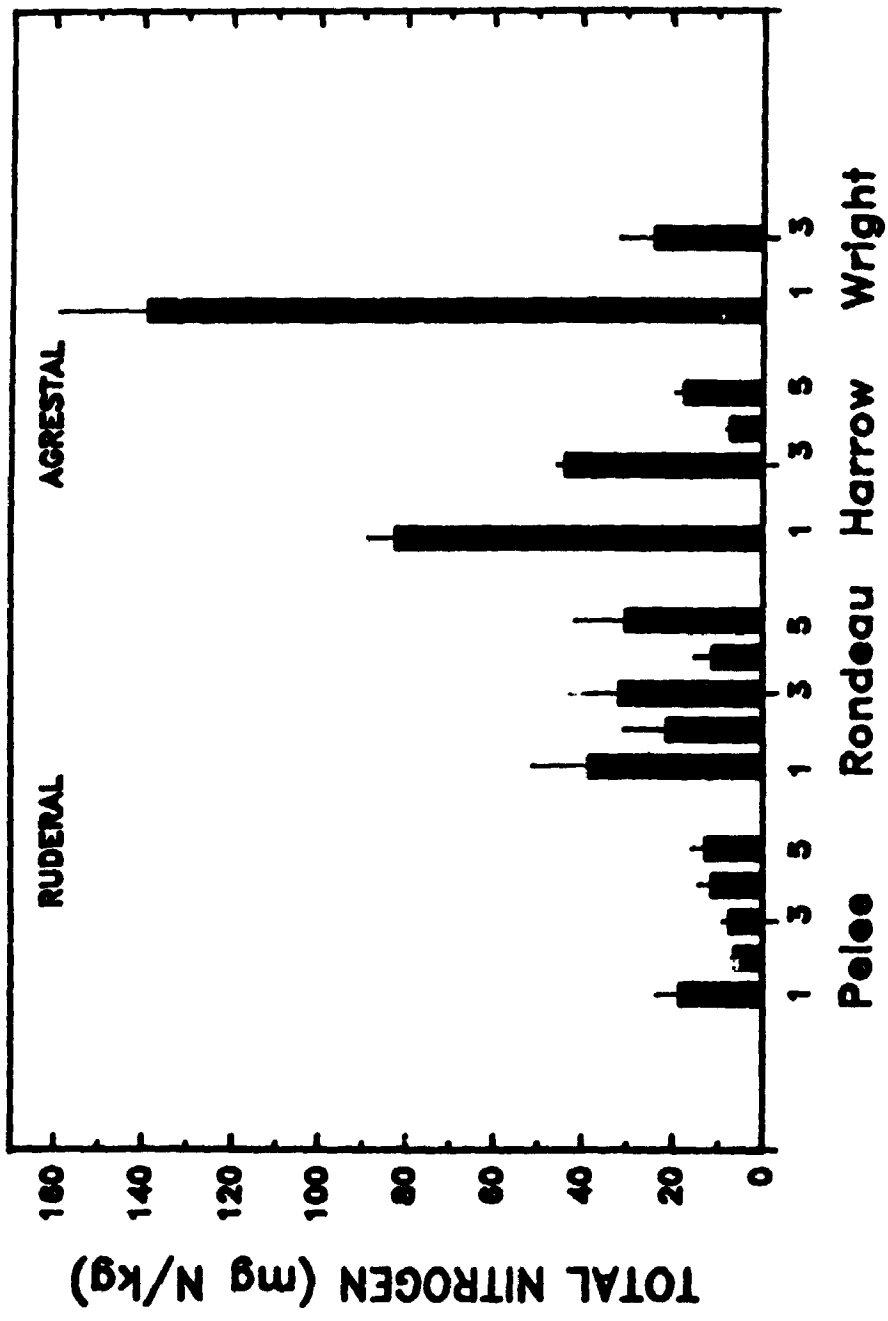
2- October 1988

3- May 1989

4- August 1989

5- October 1989.

The absence of a bar indicates that the sample was not taken.


TOTAL NITROGEN (mg N/kg)
0
20
40
60
80
100
120
140
160
RUDERAL
AGRESTAL
1 3 5 1 3 5 1 3 5 1 3 5
Palee Rondeau Harrow Wright

Regardless of the nutrient environment under which individuals were raised, the SA type (including NC) expressed a very different phenotype from that of either NA or NR types when grown in the greenhouse. Compared to northern types, the SA populations produced seedlings that were smaller at 21 days (Fig. 5.2), perhaps as a consequence of smaller seeds (Chapter 2). Juvenile plants of southern origin also took longer to produce the first dichotomous branches because of the development of greater number of leaves (i.e. nodes) subtending the split (Fig. 5.4). This subsequently delayed the switch to reproductive mode by at least a week (Fig. 5.3; in addition, at 21 days northern populations had visible floral buds). Growth habit also differed (i.e., SA upright, NA decumbent and NR almost prostrate) which was reflected in final harvest heights (Fig. 5.5; 5.2). SA plants had greater aboveground biomass at final harvest. Together with longer internodes (i.e., longer vegetative interval between formation of successive inflorescences; Fig. 5.4), these differences resulted in significantly less overall production of reproductive biomass at harvest (Fig. 5.6). Northern plants had large, ripe berries at harvest, while southern plants were only beginning to fill their berries. These phenotypes were consistent with those observed when I grew a subset of these families in agricultural fields for estimation of outcrossing rates (Chapter 2).

Table 5.2 Mixed model ANOVA for each trait in response to nutrient treatment (TRT) of EBN. Mean square values are shown, followed by the significance values of the F ratio.(P<0.05 in bold print; DF=2,2,5,10,274). The North Carolina population has not been included. See Table 5.1 for trait acronyms

TRAIT	SOURCE OF VARIATION								
	BLOCK		TRT		POP		POP*TRT		ERROR
	MS	P	MS	P	MS	P	MS	P	
HT1	0.155	**0.0085**	1.627	**0.0001**	1.360	**0.0001**	0.047	0.149	0.032
LA	0.177	0.278	7.203	**0.0001**	0.704	**0.0002**	0.176	0.241	0.137
HT2	0.958	**0.0001**	1.184	**0.0001**	3.165	**0.0001**	0.009	0.983	0.032
FL1	0.213	**0.0026**	0.078	0.067	0.252	**0.0001**	0.022	0.791	0.035
LV	0.429	0.458	2.074	**0.0333**	136.352	**0.0001**	0.426	0.651	0.546
BR	0.066	0.222	3.137	**0.0001**	1.091	**0.039**	0.686	**0.0001**	0.044
AGE1	0.069	**0.0001**	0.140	**0.0001**	0.775	**0.0001**	0.005	0.170	0.003
HT3	3.831	**0.0001**	2.443	**0.0144**	7.841	**0.0001**	0.366	**0.0001**	0.086
INTER	1.093	**0.0001**	1.017	**0.0001**	7.388	**0.0001**	0.024	0.771	0.036
BD	1.284	**0.0001**	16.487	**0.0001**	1.151	**0.0001**	0.041	**0.004**	0.015
SIDE	1.056	**0.0001**	34.232	**0.0001**	0.345	**0.0001**	0.131	**0.0001**	0.024
ANG	1.257	**0.0001**	4.426	**0.0001**	1.073	**0.0001**	0.133	**0.0001**	0.036
VDW	0.942	**0.0001**	143.824	**0.0001**	1.645	**0.0001**	0.103	**0.0164**	0.046
RPDW	4.230	**0.0001**	31.29	**0.0001**	10.484	**0.0001**	0.242	0.195	0.176
BSET	0.067	0.416	0.412	**0.0260**	0.077	0.412	0.077	0.434	0.076
BCL	0.141	**0.0306**	0.438	**0.0014**	0.153	**0.0023**	0.032	0.625	0.040
RDW	0.789	**0.0001**	1.784	**0.0001**	1.324	**0.0001**	0.059	**0.0001**	0.014
RTDW	0.006	**0.0264**	1.096	**0.0001**	0.043	**0.0001**	0.002	0.254	0.002
HAB	0.986	**0.0001**	18.930	**0.0002**	6.014	**0.0001**	0.846	**0.0001**	0.106

Fig. 5.2 Population response curves for seedling height at 21 days (HT1), leaf area (LA) and harvest height (HT3), as a function of nutrient level (1=Low; 2=Medium; 3=High). Note NC is included in this figure but not in the ANOVA (Table 5.2).

NORTHERN RUDERAL: Pelee-○ ; Rondeau-● ;

NORTHERN AGRESTAL: Harrow-▽ ; Wright-▼ ;

SOUTHERN AGRESTAL: Georgia 2-□; G1-■ ;

North Carolina-△ ;

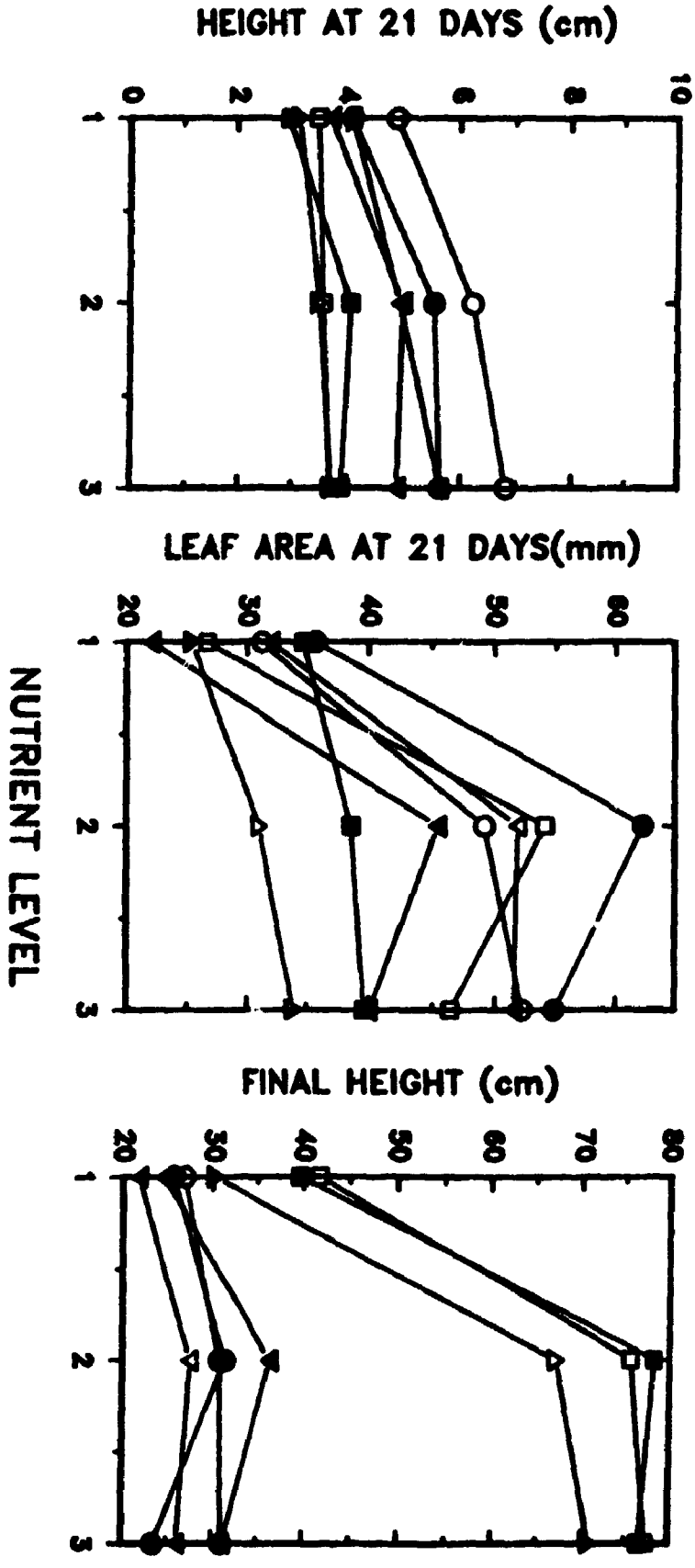

HEIGHT AT 21 DAYS (cm)
LEAF AREA AT 21 DAYS(mm)
NUTRIENT LEVEL
FINAL HEIGHT (cm)

Fig. 5.3 Population response curves of EBN for time (days) to reproductive mode (AGE1), # flowers in the first cluster (FL1), and the average number of berries in the first three clusters (BCL), as a function of nutrient level. Note NC is included in this figure but not in the ANOVA (Table 5.2). See Fig. 5.2 for symbol designations.

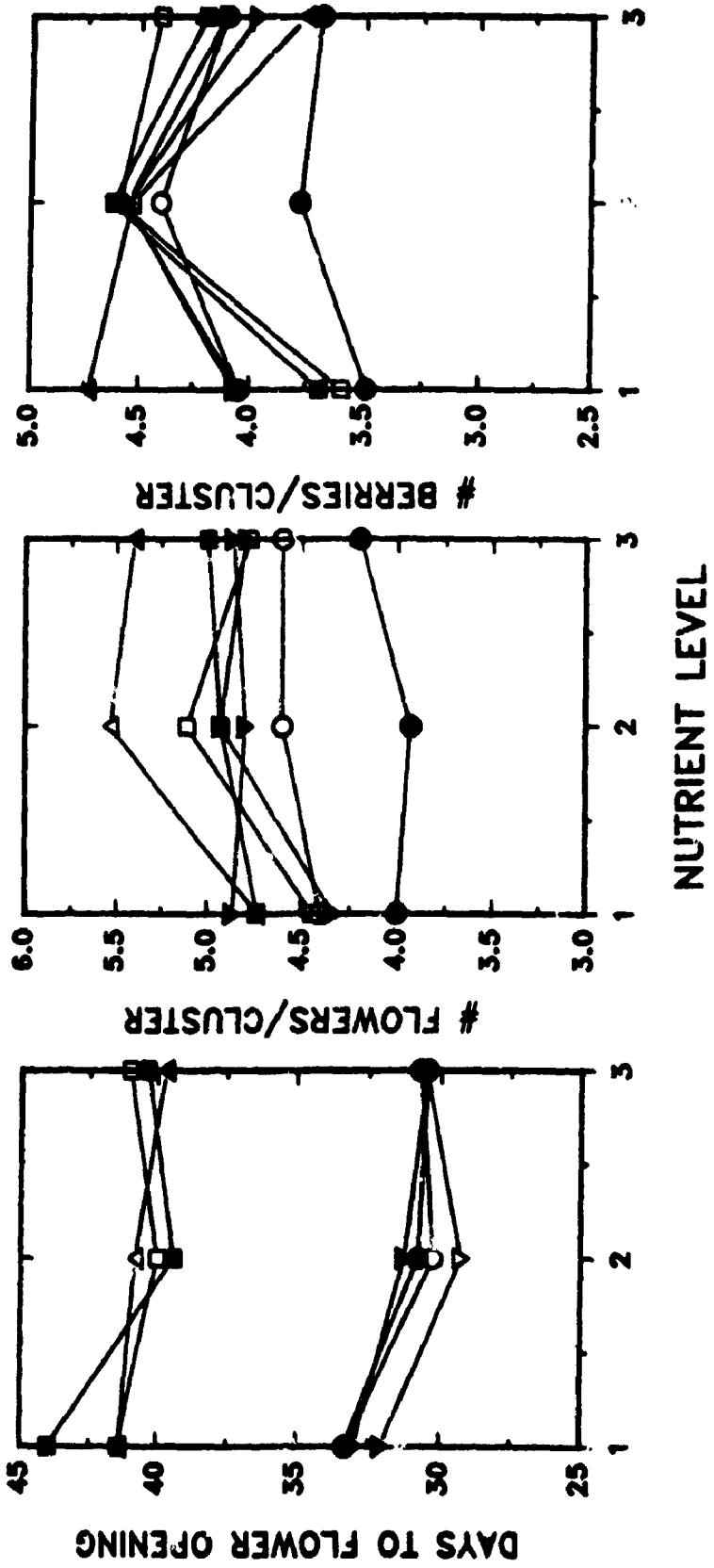

5.0
4.5
4.0
3.5
3.0
2.5
BERRIES/CLUSTER
6.0
5.5
5.0
4.5
4.0
3.5
3.0
FLOWERS/CLUSTER
45
40
35
30
25
DAYS TO FLOWER OPENING
1
2
3
NUTRIENT LEVEL

Fig. 5.4 Population response curves of EBN for number of leaves below the "split" (dichotomous branch point; LV), length of the first internode after the "split" (INTER) and the % of berries set in the first cluster (BSET), as a function of nutrient level. Note NC is included in this figure but not in the ANOVA (Table 5.2). See Fig. 5.2 for symbol designations.

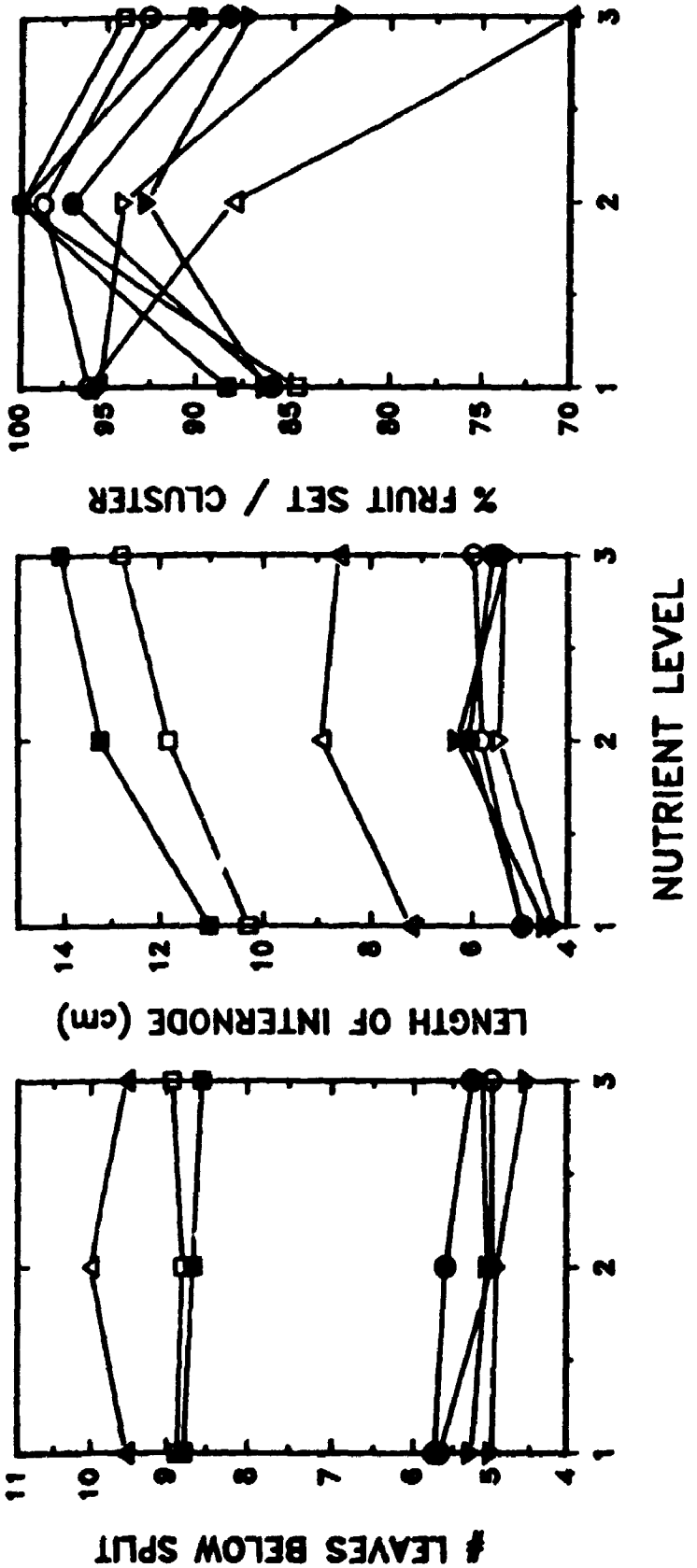

% FRUIT SET / CLUSTER
100
95
90
85
80
75
70
LENGTH OF INTERNODE (cm)
14
12
10
8
6
4
LEAVES BELOW SPLIT
11
10
9
8
7
6
5
4
NUTRIENT LEVEL
1
2
3

Fig. 5.5 Population response curves of EBN for reproductive: vegetative ratio (RDW), Root:shoot ratio (RTDW), and the growth habit (HAB) (1=upright, 3=decumbant), as a function of nutrient level. Note NC is included in this figure but not in the ANOVA (Table 5.2). See Fig. 5.2 for symbol designations.

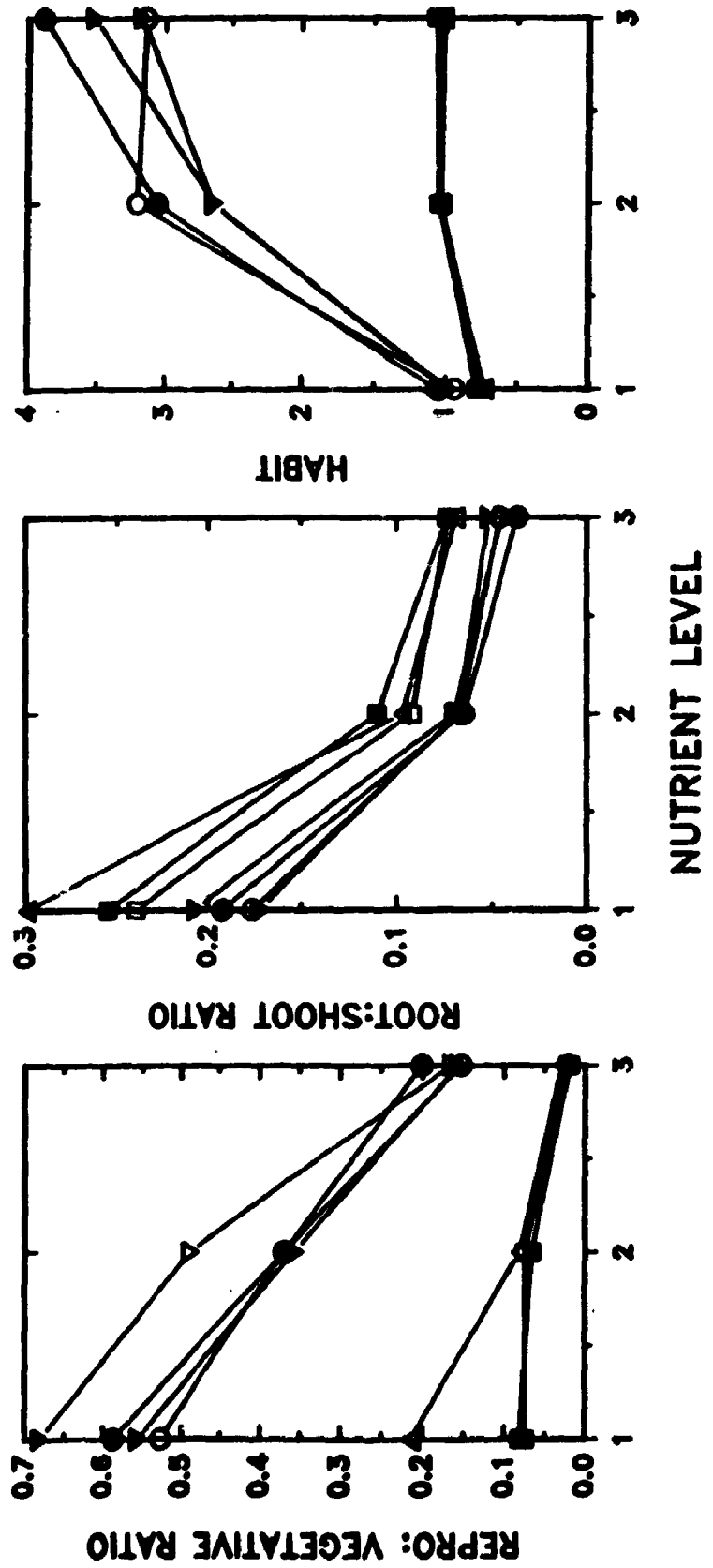
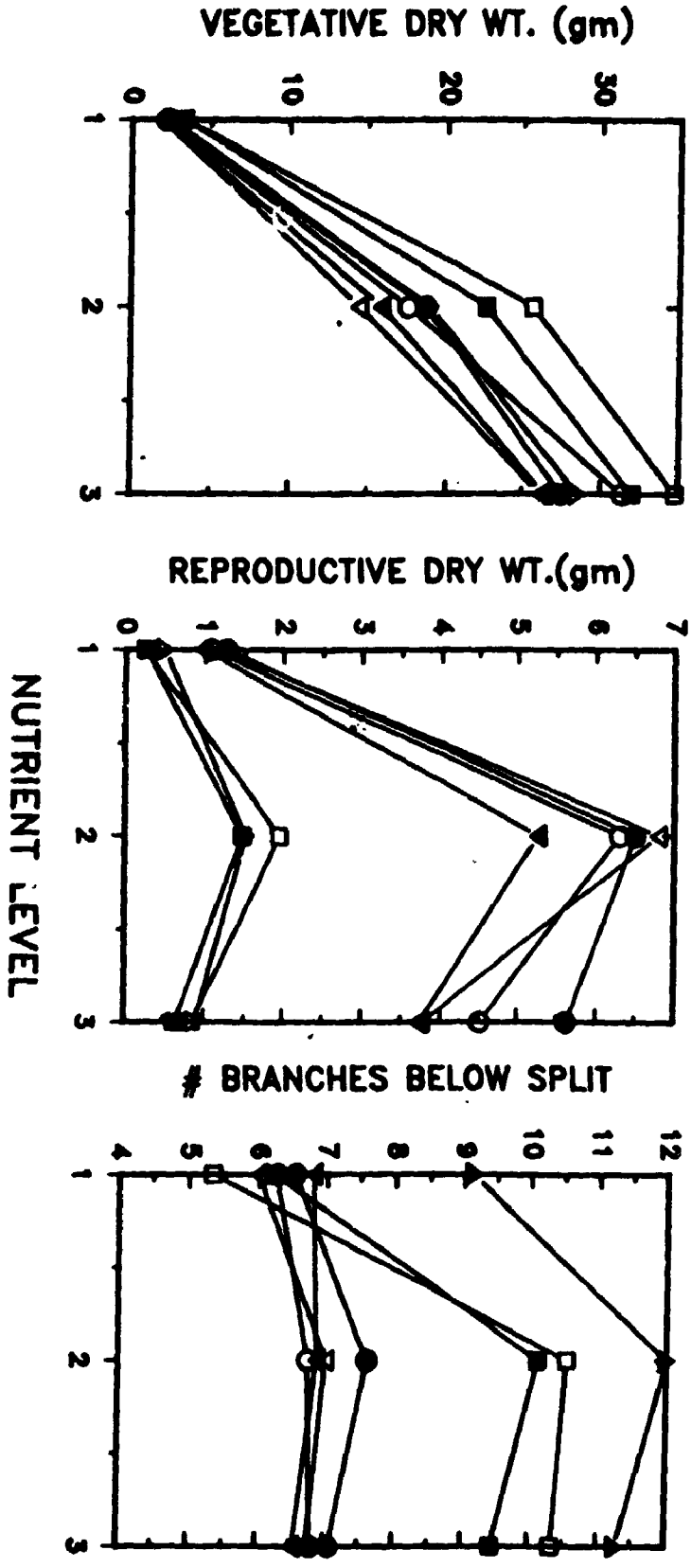

HABIT
4
3
2
1
0
ROOT:SHOOT RATIO
0.3
0.2
0.1
0.0
REPRO: VEGETATIVE RATIO
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
NUTRIENT LEVEL
1
2
3

Fig. 5.6 Population response curves of EBN for vegetative (VDW), reproductive (RPDW), and number of branches below the split (BR), as a function of nutrient level. Note NC is included in this figure but not in the ANOVA (Table 5.2). See Fig. 5.2 for symbol designations.


VEGETATIVE DRY WT. (gm)
0
10
20
30
1
2
3
REPRODUCTIVE DRY WT.(gm)
0
1
2
3
4
5
6
7
NUTRIENT LEVEL
1
2
3
BRANCHES BELOW SPLIT
4
5
6
7
8
9
10
11
12
1
2
3

The above characters which differentiate these types had larger amounts of variation partitioned among populations rather than within them (Table 5.3). Almost 100% of the total genetic variance accounted for was among populations (Ppop- Table 5.3), in the following life-history traits; age of first reproduction (AGE1), interval between successive inflorescence production (INTER) and allocation to reproduction (RPDW, RDW). Lack of population variability in the % fruit set (BSET) suggests that there are no differences in ability to self-pollinate in the greenhouse.

Based on the range of fertilizer amounts administered, the presence of highly significant amounts of plasticity in most traits, regardless of habitat origin was not surprising (TRT; Table 5.2), but direction of response was also similar in all types. Plants grown with no fertilizer added, where resources were obviously limiting, took longer to initiate flowers as a result of slower juvenile growth, had much less vegetative and reproductive tissue (a consequence of delayed reproduction) at harvest, but allocated proportionally more to reproduction than plants in non-limiting treatments (Fig. 5.2-5.6). The number of flowers produced per cluster (FL1) was fairly stable regardless of nutrient level (Fig. 5.3). Reduced berry set at low (and high) levels suggests abortion of fertilized ovaries may be reducing final yield, rather than variability in ability to set selfed seed (Chapter 2). In most populations the number of leaves (i.e. nodes) below

Table 5.3 Apportionment of genetic variation among and within populations (excluding NC) pooling all treatment levels. The percentage variance accounted for, and the significance of the F ratios are presented for populations and families nested within population. Ppop= proportion of the variance among populations / variance among families plus population. The block effect has been removed. (All F values significant at P<0.001, unless otherwise noted; DF: Pop=5, Family=22, Error=224)

TRAIT	POPULATION	FAMILY	Ppop
HT1	34.71	26.22	0.57
LA	3.59ns	9.70*	0.27
HT2	62.38	12.85	0.83
FL1	7.53ns	28.33	0.21
LV	84.89	2.56	0.97
BR	20.45	0.00ns	1.00
AGE1	79.99	1.34*	0.98
HT3	61.25	1.79ns	0.97
INTER	79.26	3.23	0.96
BD	13.86	0.00ns	1.00
SIDE	1.94	0.00ns	1.00
ANG	15.91	0.00ns	1.00
VDW	2.47	0.00ns	1.00
RPDW	33.00	0.95ns	0.97
BSET	0.00ns	25.85	0.00
BCL	1.78ns	20.77	0.08
RDW	48.27	0.00ns	1.00
RTDW	7.62	0.00ns	1.00
HAB	30.17	0.00ns	1.00

*= P<0.05; ns=non-significant

the split appears to be developmentally canalized, regardless of external environments.

The potential for differential selection among populations based on varying nutrient availability appeared to be nonexistent. Only a few traits showed significant population by environment interactions (Table 5.2), and these were generated as a result of differences in direction rather then cross-overs (i.e., proportionally larger differences between the low and medium treatment levels in the SA type (see Fig. 5.2-5.6)). At every nutrient level, with the exception of the intermediate "yield" components (# flowers/cluster, % fruit set, mean # berries/cluster), and leaf area at 21 days, the SA type was completely divergent from the northern types (all $F_{(1,22)}>6.39$). In contrast, overlap in the components of the phenotype expressed at each nutrient level between NR and NA types was extensive. Few traits differed significantly between these types, and these differences were small: Height at 21 days at medium and high fertility was greater in NR ($F_{(1,22)}>4.3$; $p<0.05$) (Fig. 5.2); NA had greater root:shoot ratios at the high nutrient level ($F_{(1,22)}=4.37$; $p=0.048$) (Fig. 5.5), and NR plants were larger (i.e., above ground vegetative biomass) at harvest in medium fertility ($F_{(1,22)}=7.54$; $p=0.012$) (Fig.5.6). In addition, given the number of traits measured, at the 5% level one would expect a small number to show significance due to error. Traits which should be directly correlated to total

lifetime reproductive output and hence fitness, such as age of first reproduction (AGE1), time between successive inflorescence openings (INTER), number of berries produced per inflorescence (BCL) and reproductive dry weight (RPDW) or allocation (RDW), showed no tendency to diverge at any nutrient level (all $F_{[1,22]}<3.98$; $p>0.05$). In a few cases, population response within the NR and NA types differed: at the medium fertility level, allocation to reproduction was higher and days to flower opening fewer, in the Harrow compared to the Wright population ($F_{[1,22]}>6.49$; $p<0.018$). Plants from the Rondeau population produced fewer berries than the Pelee plants at the medium nutrient level ($F_{[1,22]}=4.87$; $p=0.04$), a consequence of the smaller number of flowers produced (Fig. 5.3).

The phenotypes expressed by the NC plants tend to resemble those of the SA type (see figures), but show divergence in a few traits, such as decreased fruit set at higher fertilities (due to a single family which had very low fruit set), intermediate internode lengths, more branches, and higher allocation to reproduction at the low nutrient level. The NC population was not included in the analyses because the environment in which the maternal plants were raised was not the same as that for the other populations (see section 1.7.1).

The overall pattern of response to nutrients resulting from the canonical discriminant analysis did not reveal any

further differentiation of the NA and NR phenotypes (Fig. 5.7). The northern types show a high degree of overlap at all three fertility levels in the two dimensional canonical graph which accounted for 86% of the variance among types. The first canonical axis separated the low from medium and high nutrient levels, on the basis of root:shoot ratio, length of side branch and aboveground vegetative dry weight, while the second axis separated the northern and southern types on the basis of harvest height, number of leaves below the split and time to reach first reproduction. The structure coefficients used to interpret these axes (Klecka 1980), and the significance of the canonical axes are presented in Appendix F.

Not only does this analysis confirm that the NA and NR types are genetically similar, and the SA type is highly differentiated, but it also confirms that the amount and direction of plasticity in response to nutrients was similar in the northern types. The diagonal separation of the southern type at medium and high treatment levels reflects the dramatic change in the phenotype in nutrient limited situations. The distances between the northern habitat-nutrient group centroids (NR/L vs NA/L; NR/M vs NA/M; NR/H vs NA/H) are small, and non-significant (Table 5.4), while large differences are evident between low and higher nutrient levels, both for northern and southern types at each level.

Fig. 5.7 Canonical discriminant graph for individual families (each symbol) representing the nine habitat-nutrient groups

NR/L-○ ; NR/M-▽ ; NR/H-□ ;

NA/L-● ; NA/M-▼ ; NA/H-■ ;

SA/L-△ ; SA/M-▲ ; SA/H-◊ ;

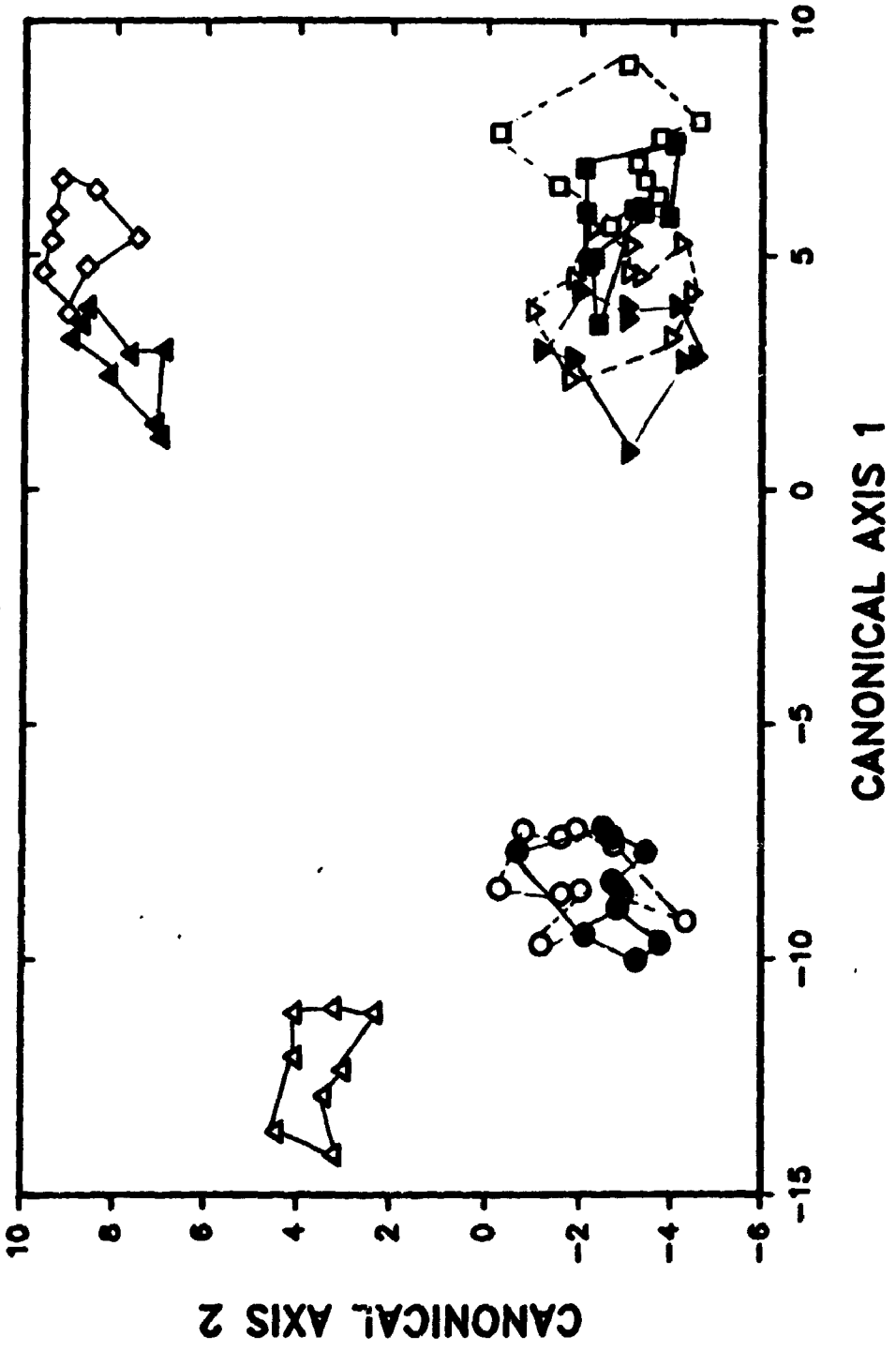

CANONICAL AXIS 2
10
8
6
4
2
0
-2
-4
-6
CANONICAL AXIS 1
10
5
0
-5
-10
-15

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Table 5.4 Mahalanobis' distance (lower half matrix) between population types (NR, NA, and SA) of EBN grown at three fertility levels (Low, Medium and High) from the canonical discriminant analysis (see Fig. 5.7), and F values (top half matrix) to test these differences. All distance measures between groups are significant ($p<0.005$) unless shown in bold print ($p>0.05$) (DF=10,9 for combinations of NR/NA and DF=10,7 with SA)

	NR/L	NA/L	SA/L	NR/M	NA/M	SA/M	NR/H	NA/H	SA/H
NR/L	--	**0.63**	22.56	45.43	36.69	44.21	64.82	52.90	59.61
NA/L	**2.53**	--	27.08	48.69	39.22	49.17	68.32	56.06	64.18
SA/L	116.01	139.27	--	68.25	63.47	42.60	85.92	77.05	58.27
NR/M	181.71	194.75	350.99	--	**1.50**	24.80	7.84	7.30	33.12
NA/M	146.74	156.89	326.44	**5.99**	--	24.51	12.26	9.49	32.71
SA/M	227.37	252.86	298.19	127.52	126.06	--	32.01	29.07	4.21
NR/H	259.29	273.27	441.90	31.35	49.05	164.60	--	**1.20**	31.80
NA/H	211.60	224.25	396.24	29.18	37.97	149.50	**4.79**	--	29.55
SA/H	306.59	330.07	407.91	170.31	168.23	29.51	163.56	151.97	--

5.3.3 Variation within populations

The six populations of EBN expressed a wide range of intra-population variability for the twelve traits analyzed, with few instances of significant family X nutrient interaction (Table 5.5; Fig. 5.8- 5.12). Populations originating from ruderal habitats appeared to be most variable, as shown by the greatest number of traits which differed significantly among families (P-9; R-11). The Wright population was most homogeneous with only two significant among family variances, height at 21 days and vegetative aboveground dry weight (Table 5.5). The Harrow population and populations from the south were intermediate to these, with 4 (G1), 6 (H) and 7 (G2) significant ratios. Traits which differed were usually the same within a type, but not necessarily between types.

Maximum among family variability was expressed at the extremes (low or high levels) of nutrient availability in most traits (Table 5.6). This lability is expressed in the intraclass correlation, indicating that the genetic component of a character is dependent on the environment in which it was calculated. Any response to selection would be greatest in the more extreme environments. The genetic component (as estimated by t) of the morphological traits varied widely, but was generally less than 0.4. Seedling height (HT1) and height at the onset of flowering (HT2) t

Table 5.5 Significance of main effects (T=Treatment; F=Family) and their interaction in the mixed ANOVA for each population to show within population variability for each trait in response to a nutrient gradient. Entries in bold print emphasize significance (P<0.05). See Table 5.1 for trait acronyms

TRAIT	POPULATION																	
	Pelee			Rondeau			Harrow			Wright			G2			G1		
	T	F	TXF	T	F	TXF	T	F	TXF	T	F	TXF	T	F	TXF	T	F	TXF
HT1	.0001	.0001	.27	.0001	.0001	.29	.0001	.0015	.96	.0001	.002	.98	.001	.95	.05	.54	.94	.37
LA	.007	.001	.22	.001	.0005	.45	.001	.006	.64	.0002	.09	.82	.0008	.76	.43	.56	.89	.50
HT2	.006	.0001	.61	.007	.0001	.34	.005	.67	.36	.0009	.43	.90	.011	.016	.11	.001	.37	.85
FL1	.72	.002	.17	.43	.008	.91	.07	.15	.59	.86	.06	.70	.67	.0001	.32	.15	.011	.65
LV	.056	.045	.51	.07	.0001	.63	.87	.11	.08	.44	.25	.25	.73	.35	.30	.25	.20	.92
AGE1	.004	.006	.18	.004	.0001	.10	.013	.04	.18	.029	.65	.45	.002	.92	.15	.049	.92	.97
HT3	.56	.0006	.28	.25	.08	.26	.07	.56	.95	.009	.88	.99	.0001	.003	.007	.001	.01	.14
INTER	.06	.11	.40	.021	.0001	.28	.012	.37	.11	.039	.81	.56	.02	.0004	.11	.06	.001	.26
VDW	.0001	.009	.67	.0001	.0004	.46	.0001	.005	.98	.0001	.029	.78	.0001	.003	.52	.0001	.046	.23
RPDW	.0001	.08	.32	.0014	.0005	.17	.0003	.002	.008	.0001	.65	.96	.0001	.048	.37	.011	.30	.24
BSET	.31	.006	.24	.59	.0001	.0001	.18	.022	.16	.65	.09	.60	.03	.50	.68	.007	.75	.98
BCL	.07	.28	.75	.43	.0001	.66	.34	.07	.017	.28	.07	.35	.058	.001	.055	.06	.28	.61

Fig. 5.8 Norm of reaction plots of each population (excluding NC; see Fig. 5.9) to show variation among five (or three for G1) families for seedling height as a function of nutrient level. Symbols denote individual families.

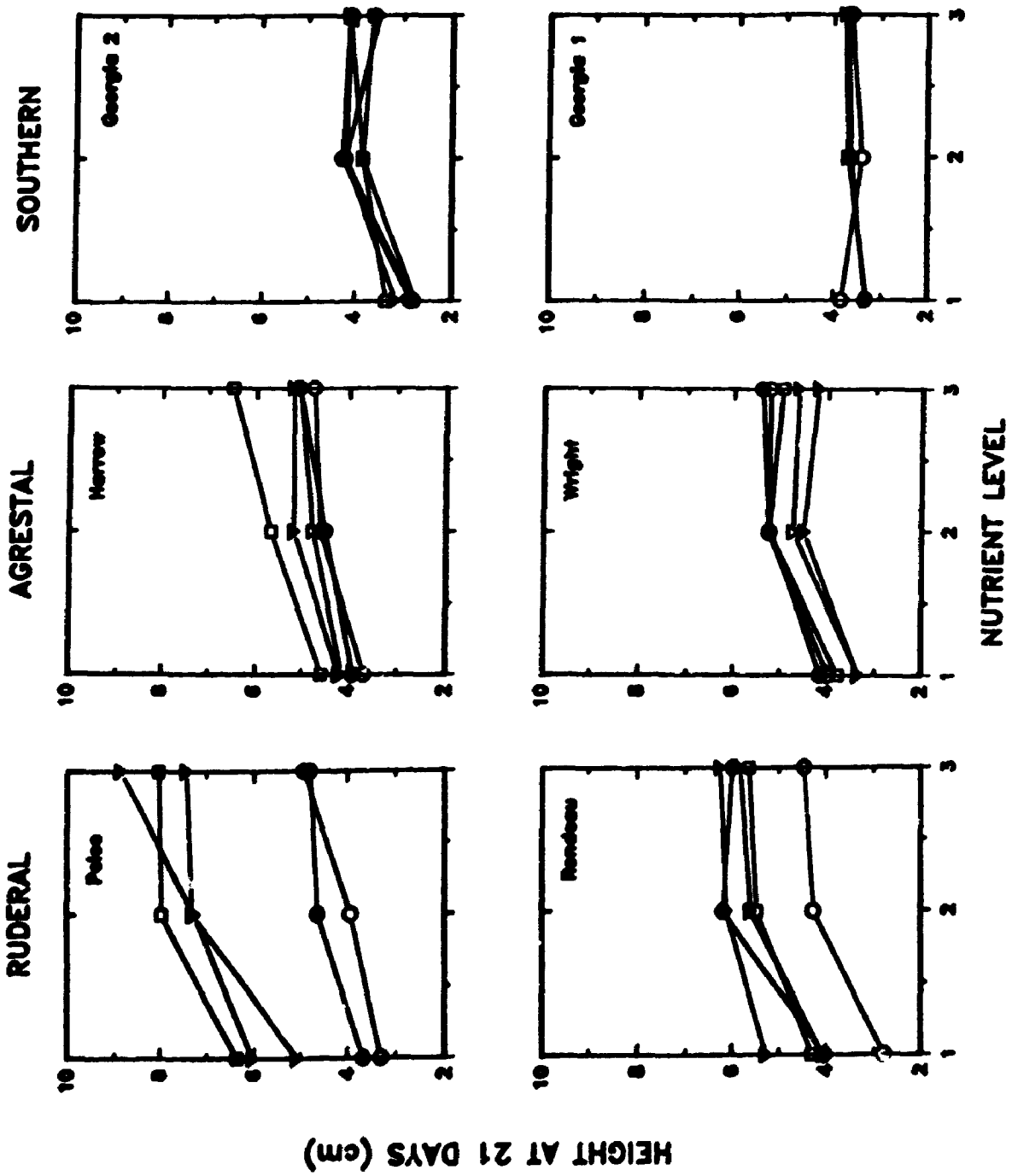

SOUTHERN
AGRESTAL
RUDERAL
Georgia 2
Georgia 1
Narrow
Wright
Pulse
Bandana
HEIGHT AT 21 DAYS (cm)
NUTRIENT LEVEL

Fig. 5.9 Norm of reaction plots of the North Carolina population showing the five families for seedling height, number of days to first flower opening and the average number of berries in the first three clusters as a function of nutrient level.

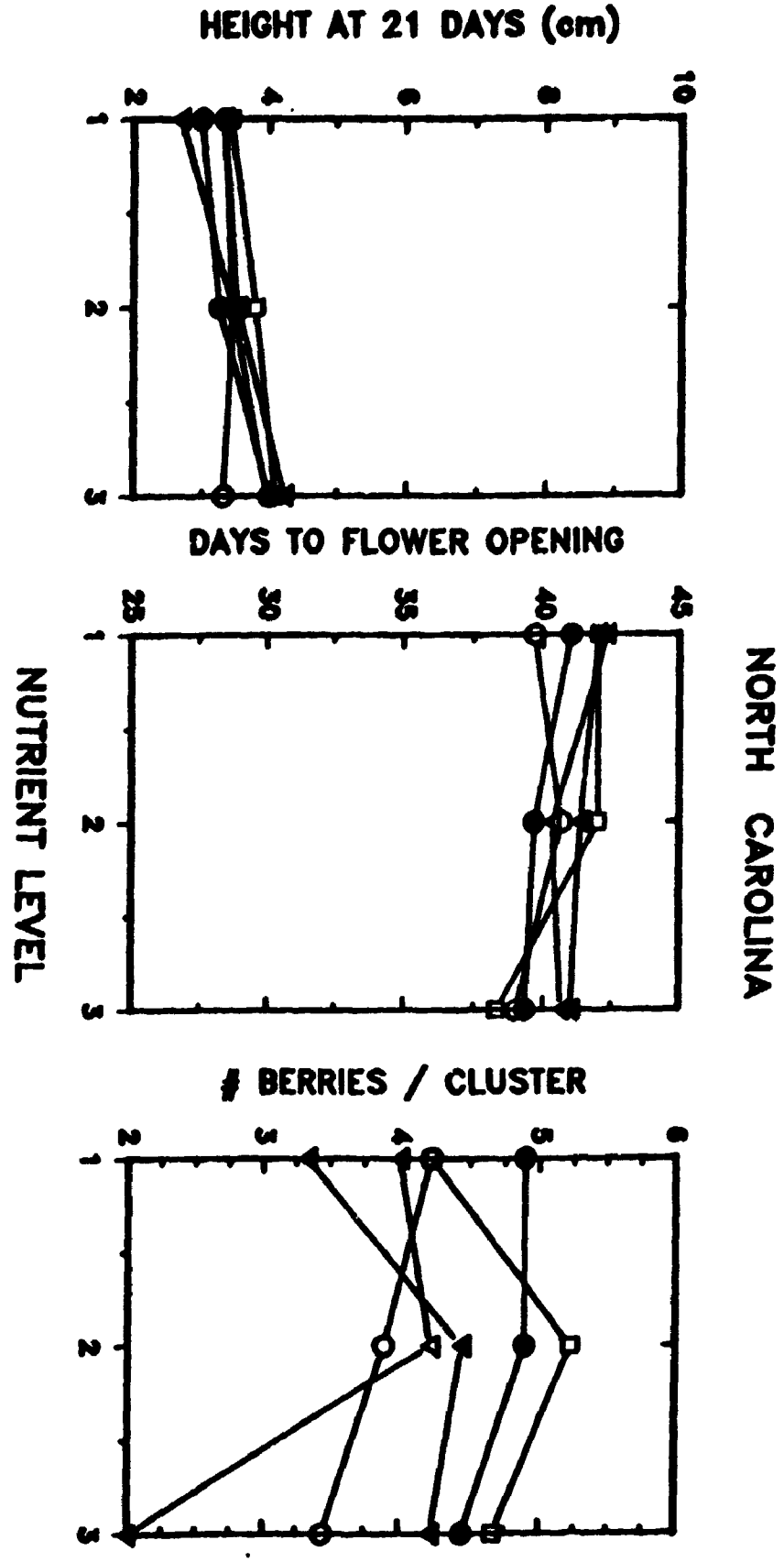

HEIGHT AT 21 DAYS (cm)
DAYS TO FLOWER OPENING
BERRIES / CLUSTER
NUTRIENT LEVEL
NORTH CAROLINA

Fig. 5.10 Norm of reaction plots of each population (excluding NC; see Fig. 5.9) to show variation among five (or three for G1) families for number of days to first flower opening as a function of nutrient level. Note that the vertical axis is different for the southern populations.

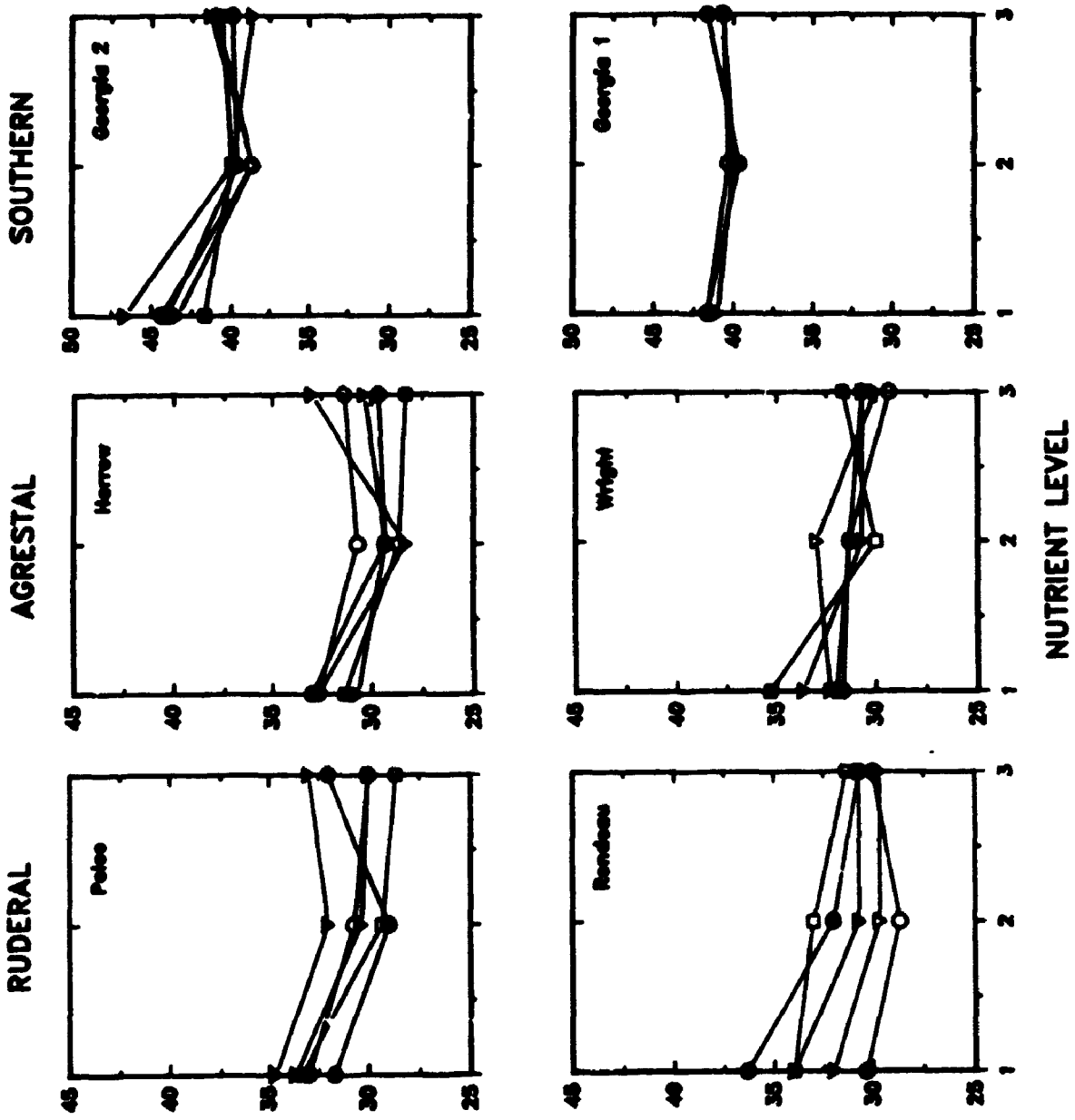

SOUTHERN
AGRESTAL
RUDERAL
Georgia 2
Georgia 1
Norrow
Wright
Poke
Rondeau
NUTRIENT LEVEL
DAYS TO FLOWER OPENING

Fig. 5.11 Norm of reaction plots of each population (excluding NC) to show variation among five (or three for G1) families for harvest height as a function of nutrient level. Note that the vertical axis is different for the southern populations.

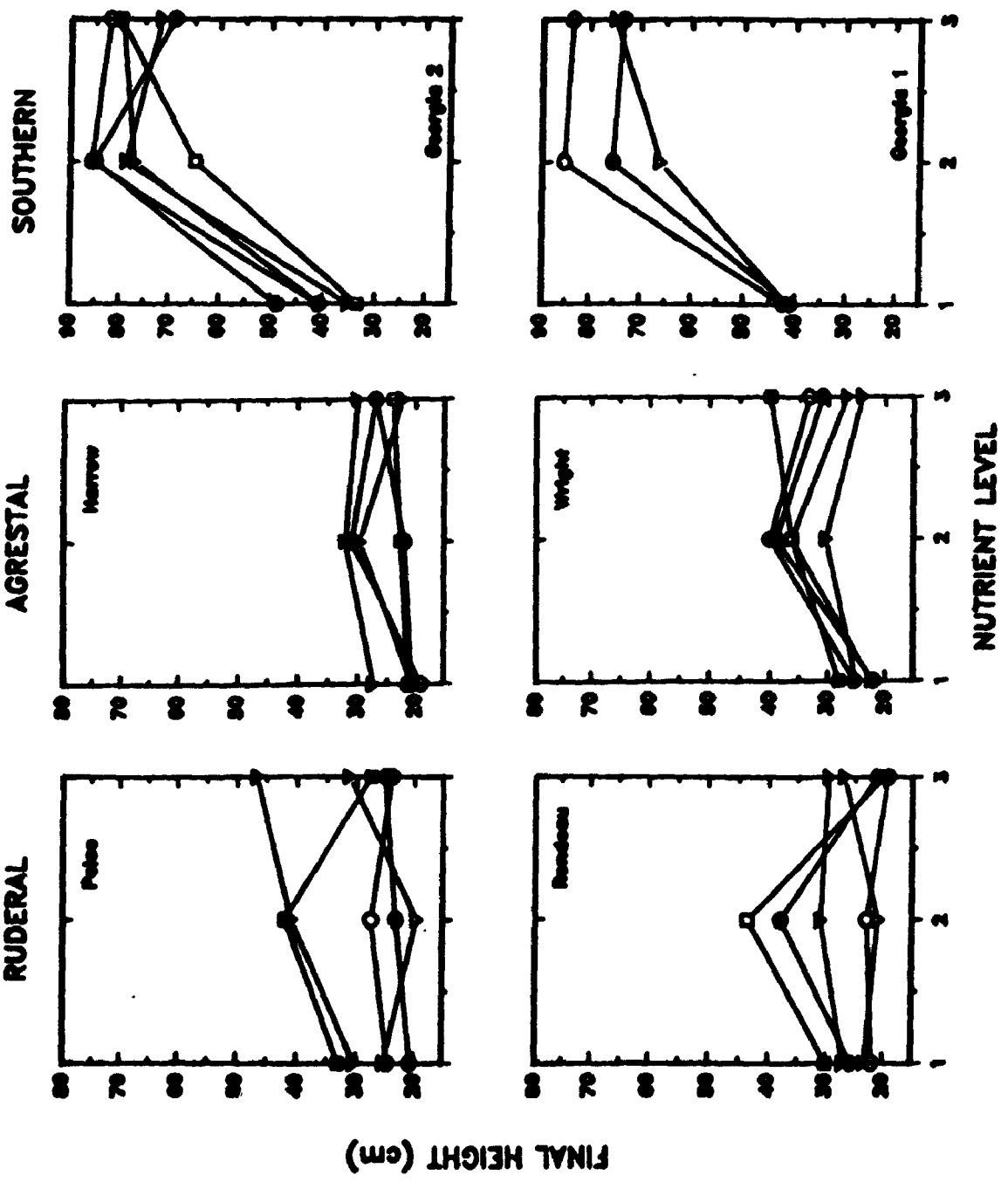

SOUTHERN
AGRESTAL
RUDERAL
Georgia 2
Georgia 1
Narrow
Wright
Peiso
Random
NUTRIENT LEVEL
FINAL HEIGHT (cm)

Fig. 5.12 Norm of reaction plots of each population (excluding NC; see Fig. 5.8) to show variation among five (or three for G1) families for average number of berries set in the first three clusters, as a function of nutrient level. Note that the vertical axis is different for the southern populations.

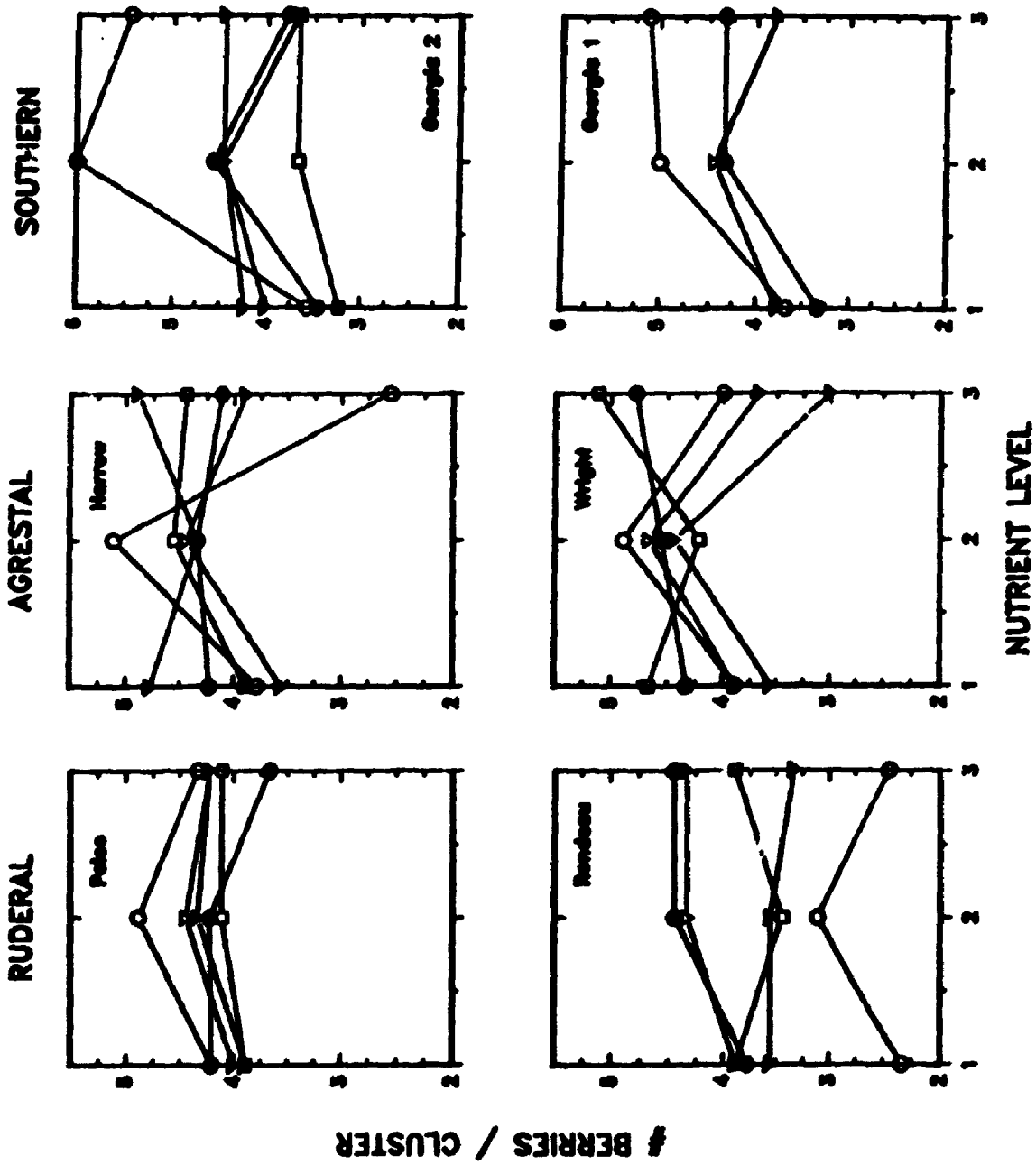

SOUTHERN
AGRESTAL
RUDERAL
Georgia 2
Georgia 1
Norrow
Wright
Peiko
Rondeau
NUTRIENT LEVEL
BERRIES / CLUSTER

Table 5.6 Apportionment of genetic variation among and within populations (excluding NC) for each nutrient level. Percentage variance accounted for, and the significance of the F ratios are presented for populations and families nested within population. The block effect has been removed. (All F values significant at P<0.001, unless otherwise noted; DF=5,22,56). t=intraclass correlation coefficient (see text for explanation)

TRAIT	SOURCE	NUTRIENT LEVEL 1	2	3
HT1	POP	32.91	46.05	55.70
	FAM	40.56	40.13	27.07
	t	.604	.744	.611
LA		5.28ns	16.44*	3.58ns
		15.10ns	16.47ns	24.49*
		.159	.197	.254
HT2		62.60	66.48	73.58
		21.18	18.90	4.35ns
		.566	.564	.165
FL1		15.40*	10.53ns	0.00ns
		4.99ns	31.96*	33.94*
		.059	.357	.339
LV		81.00	86.75	86.82
		7.52	1.90ns	1.20ns
		.396	.145	.091
BR		8.56ns	78.98	67.01
		7.74ns	0.95ns	0.00ns
		.045	.045	.000
AGE1		80.94	87.21	87.18
		3.00ns	2.86*	3.46*
		.157	.224	.270
HT3		60.25	65.37	74.89
		4.23ns	3.63ns	0.00ns
		.106	.105	.000

* P<0.05; ns=non-significant

continued.....

Table 5.6 continued

TRAIT	SOURCE	NUTRIENT LEVEL 1	2	3
INTER	POP	84.66	73.89	86.36
	FAM	7.25	7.49*	0.00ns
	t	.473	.287	.000
BD		55.74	64.16	70.12
		14.77*	0.00ns	1.35ns
		.334	.000	.045
SIDE		43.12	25.24*	9.74ns
		16.27*	4.85ns	8.63ns
		.286	.065	.096
ANG		13.17*	35.95	32.86
		0.00ns	4.59ns	4.14ns
		.000	.072	.062
VDW		56.48	58.27	15.29*
		14.69*	13.33*	14.44ns
		.338	.319	.170
RPDW		75.78	67.89	42.62
		0.00ns	9.76*	19.14*
		.000	.304	.333
BSET		0.00ns	8.08ns	0.00ns
		30.39	6.11ns	44.70
		.304	.066	.447
BCL		5 24ns	10.96ns	0.00ns
		19.21*	13.57ns	43.30
		.203	.152	.433
RDW		76.62	73.19	44.96
		0.00ns	4.65ns	11.25*
		.000	.173	.204
RTDW		30.35	38.55	62.41
		5.01ns	4.71ns	5.05ns
		.072	.077	.134
HAB		14.15*	55.11	89.93
		15.62ns	1.84ns	6.90ns
		.182	.041	.682

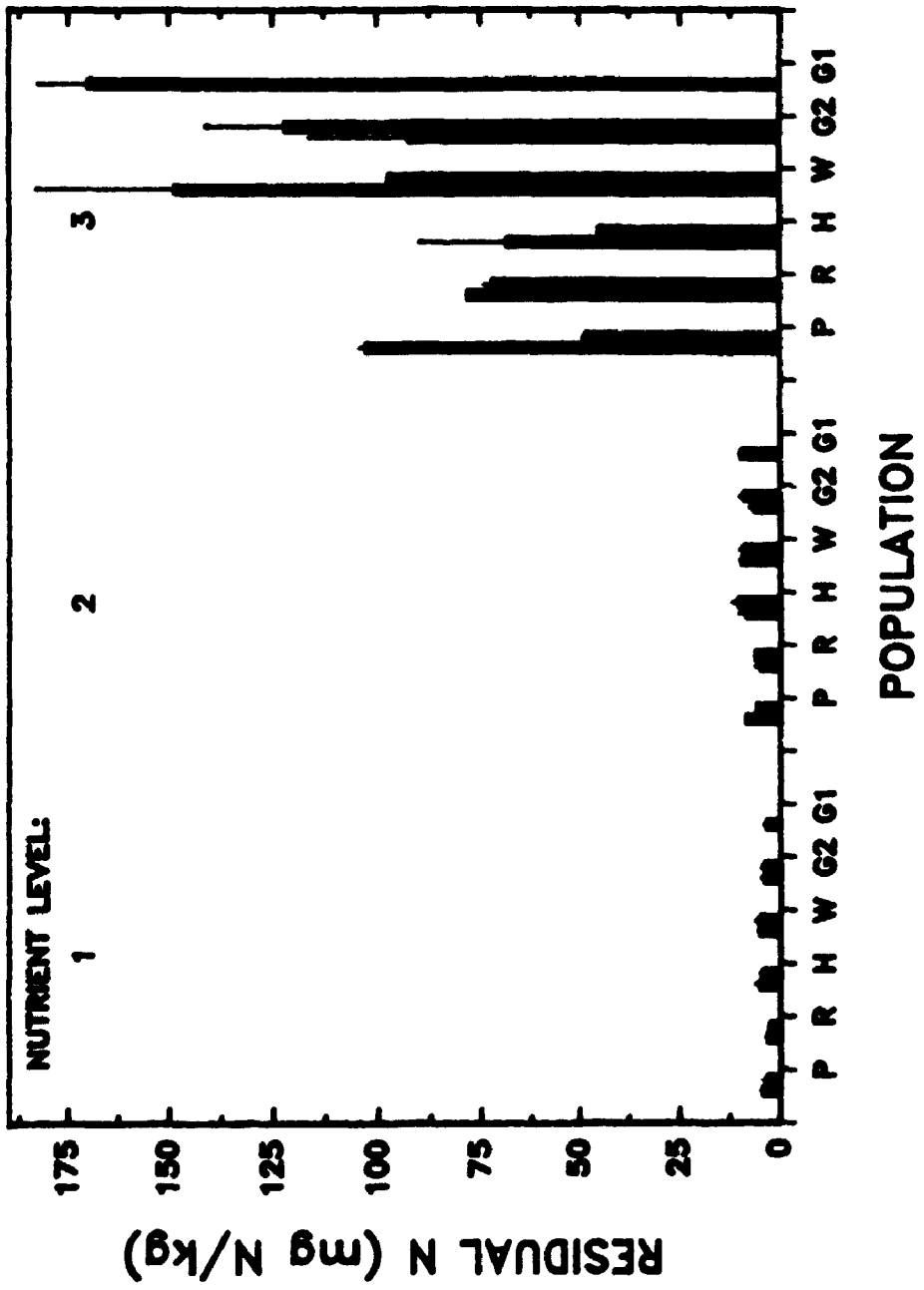
values were above 0.55 (Table 5.6). Traits which comprise components of fitness (AGE1, INTER, FL1, BCL) tended to have smaller t values, suggesting limited ability to respond to selective pressures.

There are several reasons for interpreting these t values with caution. The amount of variance sampled with only 5 families may underestimate the total represented in each population and hence minimize the potential for selection to act. These estimates were generated from widely divergent populations which were pooled, resulting in an "average" t value for all the populations (Lawrence 1984). Given the differences in family variance encountered among populations (Table 5.5), and in other studies (Venable 1984; Venable and Burquez 1989) which have shown population differences in t values, it is probable that these t values would also vary with the population. Unfortunately the replication at each treatment level was insufficient to obtain reliable estimates for each population.

5.3.4 Variation in residual N

It was obvious that the growth of EBN was severely limited with no additional input of fertilizer, but all families were still able to produce flowers. All families were able to extract all available N from the soil (Fig. 5.13). It appears that the medium amount of fertilizer was approaching a limiting condition. All populations were able to utilize more than 95% of the 200 mg/kg of N that was

Fig. 5.13 Total residual nitrogen remaining at the termination of the nutrient experiment. Each bar represents the mean of a family pooled across blocks within each population, at each of treatment level (1=Low; 2=Medium; 3=High).


RESIDUAL N (mg N/kg)
175
150
125
100
75
50
25
0
NUTRIENT LEVEL:
1
2
3
P R H W G2 G1
POPULATION

applied over the duration of the experiment. At the "luxurious" rate, the ruderal and Harrow populations were able to harvest more of the N (87-90%) than the southern and Wright populations (71-82%), but there was substantial variability between the 2 families sampled within the populations in ability to harvest this N (Fig. 5.13).

The question of interest is, given the inter- and intra-population variability in uptake of N, was the same variability expressed in the production of biomass? The severe limitation imposed at the low level showed no relationship between vegetative biomass and residual N, with all populations producing minimal amounts (Fig. 5.14). However, at the medium resource state, both the vegetative and reproductive biomass (Fig. 5.15) reflected the obvious north-south differences seen in the last section (see Fig. 5.6). The lack of relationship between residual N and vegetative biomass suggests that there is a limit to the amount of N that can be utilized by EBN under greenhouse conditions, and that efficiencies in the conversion of nutrients to biomass may vary between and within populations. At the high level of fertilizer application insufficient levels of the other macronutrients may have limited growth. The decrease in reproductive biomass at the luxurious level reflects the tendency of members of the Solanaceae to accumulate vegetative biomass at high levels of N, at the expense of reproductive function.

Fig. 5.14 The aboveground vegetative biomass (dry weight) plotted as a function of the residual nitrogen. Nutrient levels (1, 2, 3) are indicated adjacent to data points. Each symbol represents the mean of the families presented in Fig. 5.13. Population symbols as in Fig. 5.2.

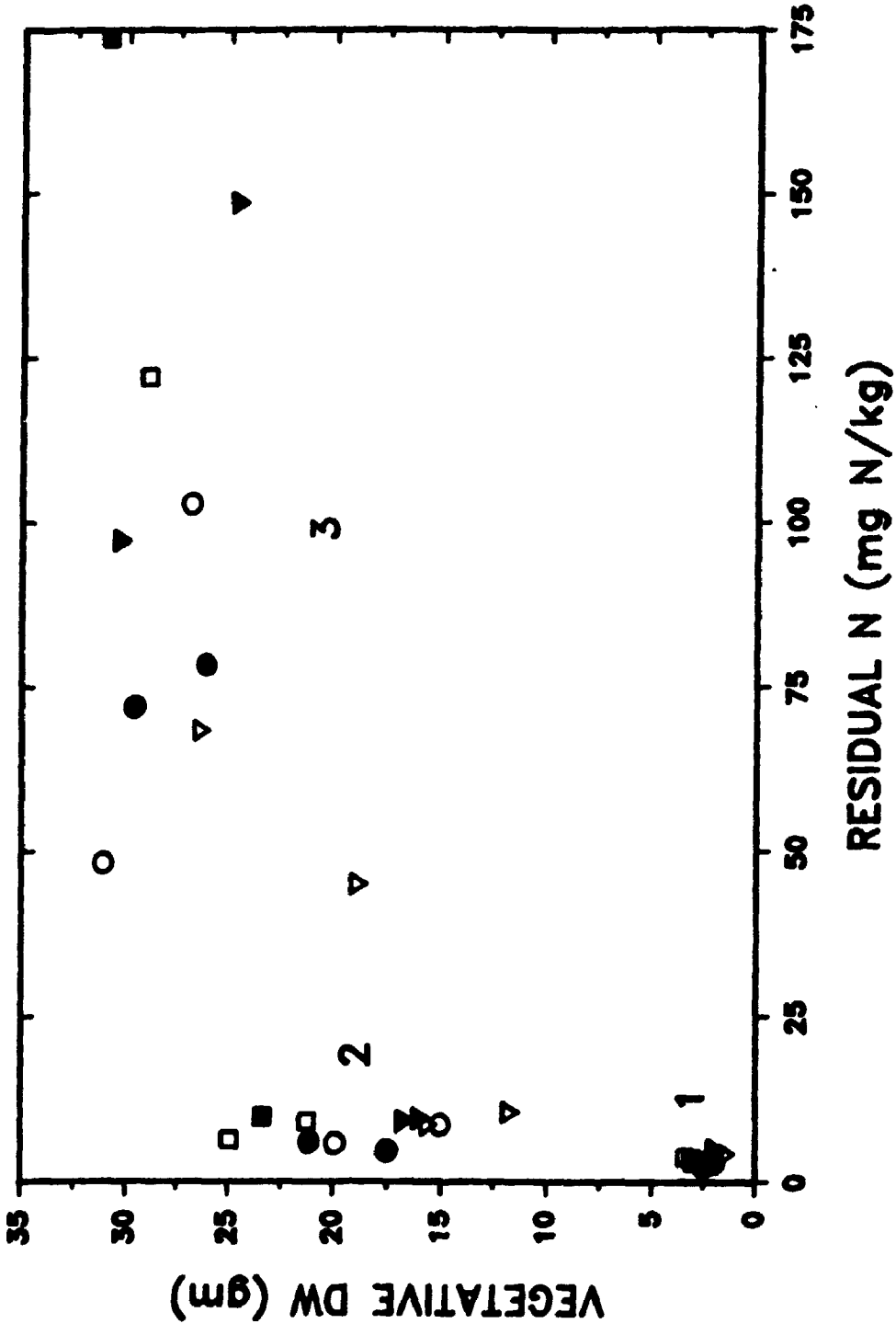
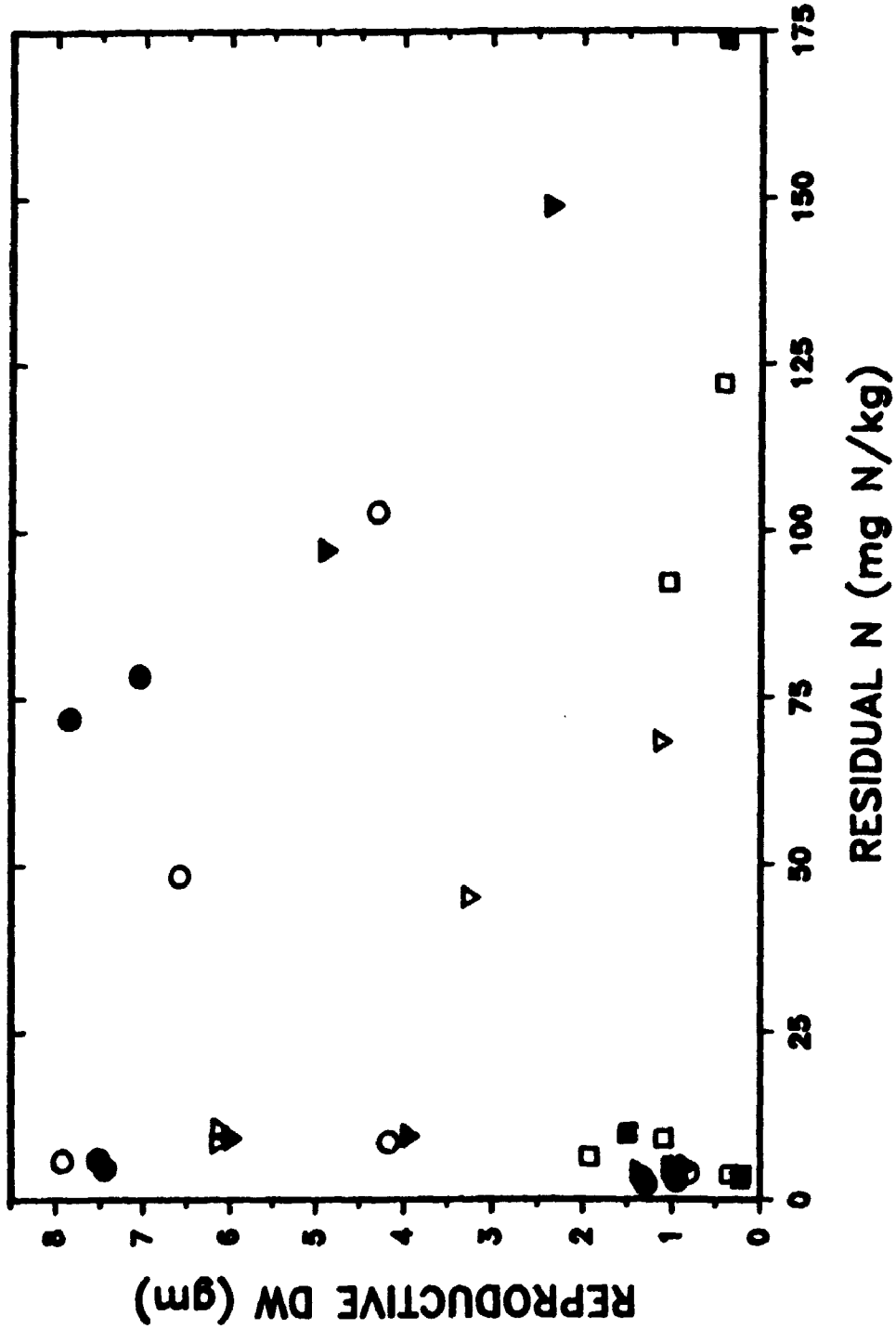

VEGETATIVE DW (gm)
RESIDUAL N (mg N/kg)
35
30
25
20
15
10
5
0
175
150
125
100
75
50
25
0
1
2
3

Fig. 5.15 The reproductive biomass (dry weight) plotted as a function of the residual nitrogen. Symbols as in Fig. 5.2.


REPRODUCTIVE DW (gm)
RESIDUAL N (mg N/kg)
0
1
2
3
4
5
6
7
8
0
25
50
75
100
125
150
175

5.4 Discussion

It is obvious from the degree of phenotypic resemblance among northern populations, regardless of habitat of origin (ruderal or agrestal) or nutrient availability, that there has not been rapid genetic differentiation associated with high input agroecosystems. Nor was there divergence in the amount or direction of the genotype by nutrient interaction in yield. Both types were able to maximize reproductive output in proportion to nutrient availability. If relative fitness is evaluated by the amount of reproductive biomass at harvest (RPDW) or allocation to reproduction (RDW), individuals from "high" input habitats (NA) showed no increase over that produced by individuals from the "lower" input habitats (NR), nor is there evidence of decreased reproductive output of NA under very limiting nutrient conditions.

Cocklebur (Xanthium strumarium) populations originating from ruderal (low fertility) or native (high fertility) sites showed complete overlap in phenotype at high and medium resource levels, but contrary to EBN, showed significant divergence at low resource availability (Blais and Lechowicz 1989) (note: agrestal populations were not included in this study). Few other studies have specifically tested differentiation in response to nutrient availability in agrestal habitats (Warwick 1990a). Echinochloa colonum individuals from low fertility habitats

had lower seed output in high treatments than low or medium treatments, and higher root:shoot ratios at all levels than plants orginating in high fertility habitats (Kapoor and Ramakrishnan 1974). In EBN, allocation to roots was strongly nutrient dependent in all populations, especially in low and medium treatments. Plants from nutrient poor habitats do not show less plasticity in root:shoot ratios than plants from richer habitats (Chapin 1980). Sobey (1987) found differences in age of first reproduction and seed size between Stellaria media plants collected from gardens and gull colonies, but these were independent of soil nutrients.

Several studies (see Baker 1974; Barrett 1988) have detected a trend towards decreasing age at first reproduction in populations associated with agricultural systems (although see Price and Kahler 1983 for the opposite trend). This has not been the case in EBN. All 4 northern populations, regardless of habitat of origin or nutrient conditions of culture, reached reproductive maturity in roughly the same time period.

All northern populations expressed very plastic phenotypes which would result in potentially broad resource niches in nature. Such levels of phenotypic plasticity may not be surprising given the variability in available nitrogen associated with both of these habitats. Parrish and Bazzaz (1985) suggested this is evidence for convergence

in adaptation to a variable environment. Many of the recent studies have detected significant population differences in amount or direction of plasticity (Wilken 1974; Schwaegerle and Bazzaz 1987; Lotz and Blom 1986; MacDonald and Chinnappa 1989; Schlichting and Levin 1990). However most of these studies have dealt with native, non-weedy species. The lack of divergence in plastic response may result from large-scale environmental variability within sites compared with between sites, lack of sufficient genetic variation or negative genetic correlations among traits (Schlichting and Levin 1990).

The extensive similarity in key life history traits indicate that agrestal populations of EBN are not a genetically discrete subset of ruderal genotypes (or vice versa), but reflect selection for extensive phenotypic plasticity (Blais and Lechowicz 1989). Successful colonization of northern habitats appears to be via a "general purpose" genotype (Baker 1974) or "individual buffering" (Moran et al. 1981) rather than by the formation of specialized "agroecotypes" ("population buffering") in response to nutrient variability. Wu and Jain 1978, Moran et al. (1981) and Blais and Lechowicz (1989) also detected ample amounts of phenotypic plasticity in widespread weedy species in response to environmental gradients.

The lack of differentiation detected in the genetic and plastic components of response to nutrient level between NA

and NR types may have been due to the failure to include other yield components that contribute to total fitness in agroecosystems in the analyses. As a consequence of development, the predicted sequence of response of yield components to stress (including nutrient stress) would be: fruit number, seeds per fruit and lastly seed weight (Harper, Lovell and Moore 1970). The first two components determine seed quantity, the latter seed quality. One of these components was directly measured (number of berries set per cluster), and it responded in a similar manner regardless of population origin or nutrient level (Fig. 5.3). Plasticity in seed number per fruit was not measured, but northern (both NA and NR) populations differed by only 10% in the mean number of seeds set per berry when grown in agrestal habitats (Appendix C). Altnough the response of seed weight to nutrient availability was not measured, the majority of seed weight variability encountered in the S_1 plants was between the northern and southern populations (section 3.3), rather than between NA and NR types. There is the possibility that the NA and NR types expressed different patterns of plasticity in response to nutrient stress in their adjustments of these yield components (Marshall, Levin and Fowler 1986), but the lack of plasticity in fruits per cluster, and the relatively limited variation between types in the other two components suggests this is unlikely.

It is obvious that the SA type of EBN shows significant divergence in life history traits from the EBN currently colonizing northern agrestal habitats. The much shorter pre-reproductive period of the northern plants corresponds with the reduced number of frost free days in SW Ontario compared to NC or GA (170 vs 220/250). Potvin (1986) found a similar decrease in age to first reproduction, and increase in allocation to reproduction in the annual selfer Echinochloa crus-galli (barnyard grass) in reciprocal transplant experiments between Quebec and North Carolina.

It appears that southern populations would be unable to successfully colonize northern agroecosystems due to a developmentally canalized growth pattern causing delayed reproduction, and longer vegetative intervals between the formation of inflorescences resulting in reduced lifetime reproductive output. The low t values of LV, AGE1 and INTER (assuming a proportion is additive genetic variation) suggest there would be limited ability for the southern populations to respond to directional selection for shorter pre-reproductive time which is imposed by agricultural practises associated with "short" term crops such as tomato varieties which are harvested in 75-85 days. In later maturing tomato varieties (e.g., Heinz 722; 90-100 days) this limitation may be circumvented by the direct transplantation of juvenile SA plants at the verge of reproductive maturity, such that southern individuals are

able to produce viable seeds in this time period (see section 1.6). If tomatoes, or other longer maturing crops (e.g., soybeans) were grown in the following season, lack of sufficient growth time and/or acute susceptibility to herbicides (Chapter 4) would presumably select against southern genotypes in the next year.

If a number of characters are considered, ruderal populations of EBN appeared to be more genetically heterogeneous than the agrestal types (both southern and northern), but populations within types vary widely. A similar range of differences between populations was also found in the weed Datura stramonium (Weaver et al. 1985). Differences in genetic variability among populations may be derived from non-adaptive sources such as drift and founder effect, and/or variability in outcrossing.

Experiments which attempt to partition genetic from environmental effects on phenotypic variation in controlled environments are often dismissed as being meaningless (Endler 1986), because genetically based differences are often overwhelmed under field conditions (Venable 1984). The lack of significant population, or family by nutrient interactions in most traits suggested that the documented differences would be detected under "natural" or field conditions (Venable and Burquez 1989).

CHAPTER 6-SUMMARY AND CONCLUSION

This study underscores the importance of evaluating population variability and plasticity of life-history traits in a number of potentially important selective environments which may contribute to population differentiation. The predictions of reduced genetic variability and increased plasticity in northern agrestal habitats of eastern black nightshade due to environmental homogeneity, marginal location, and/or recent establishment were not realized. Under a wide range of potentially diversifying conditions, northern agrestal populations were not genetically depauperate compared with ruderal, or southern/central populations. In addition, the expression of significant plasticity in all traits has not acted to buffer the divergence of mean trait response from directional selection encountered in agrestal habitats. Over a wide range of environments, the amount of plasticity of a trait was independent of its mean response (i.e., the level of genetic variability was not tied to expression of plasticity), showing that the two components can evolve separately (Schlichting 1986; MacDonald and Chinappa 1989). Even with significant plasticity, population differentiation was detected in life-history characters in predicted directions associated with habitat of origin (i.e. delayed reproduction in southern habitats).

The expression of broadly tolerant phenotypes by all

families of each habitat type, with the ability to reproduce across a wide gradient of conditions is consistent with the "general purpose genotype" associated with many weeds (Baker 1974), and argues against the presence of "agrestal" genotypes which specialize in the colonization of agroecosystems. Such high levels of plasticity are consistent with that found in other annual selfing weeds (Wu and Jain 1978; Moran et al. 1981; Blais and Lechowicz 1989). The expression of variable amounts of divergence in life history traits associated with germination syndromes (NA vs NR), herbicide tolerance (NA vs SA) and fecundity (north vs south) was dependent on environment. Expression of a similar phenotype in most of the environments tested underlines the importance of selection and maintenance of plasticity in the adaptation of weedy selfing annuals to various environmental regimes.

This study allows the prediction of the net effect of each of the tested environments on fitness, and hence the potential for invasion of agroecosystems. Firstly, mortality selection may act via various abiotic factors against both ruderal and southern agrestals in northern agroecosystems. At germination, selection against earlier emergence of the ruderal families imposed by plowing and cultivation may result in higher levels of mortality, compared with agrestal families (Chapter 3). The detection of variability in germination rate within the ruderal

populations may allow a response to such directional selection pressures, with the evolution of delayed germination (Chapter 3). Other components of the germination syndrome, such as the light and/or depth requirements must also be fulfilled in the germination process. This buffering capacity could act to shelter ruderal seed in the seed bank, thereby circumventing the directional selection. At emergence, further selection against the ruderal families would occur when herbicide rates were high, due to increased susceptibility (Chapter 4). In addition, differences in selection may act through differences in fertility, as a result of herbicide application and competition if reproductive output is concomitantly depressed in ruderals. Selection against ruderals in key adult fitness characters (e.g., age to first flowering and reproductive output) would not be predicted as they did not diverge from agrestals under variable fertility (nutrient) regimes (Chapter 5).

Selection against the southern agrestal colonists in northern agroecosystems would act at different stages compared with those of ruderal populations. Emergence would be synchronous, given the similar response to temperature of northern and southern agrestal populations (Chapter 3). At the seedling stage, relatively higher levels of mortality would be predicted for Georgia agrestal families, even at low rates of metribuzin application (Chapter 4). This

greater susceptibility to metribuzin may be a function of smaller seeds (Chapter 3) which produce smaller seedlings, and lower growth rates found in southern families, compared to northern families (Chapter 4 and 5). The NC families were more tolerant to the applied herbicide which would presumably increase survival in northern tomato crops (Chapter 4). And finally, it is predicted that fertility selection would exclude the southern families (regardless of enzyme genotype) from northern short-season agroecosystems due to delayed onset of reproduction and the inability to produce viable seeds (Chapter 5). Successful reproduction would occur if weeds of southern origin (NC) were transplanted into tomato fields (Chapter 1), but viable seeds may not be produced the following year.

Evaluation of these predictions could be carried out by seeding families of NR, SA and NA types into a short-season crop (tomato) and a long-season crop (soybeans). The impact of each of these divergent traits on the fitness of each of the families in these diverse agrestal habitats could then be determined at each life stage. As the selective potential of these environmental regimes could differ in intensity and direction over the life of EBN, separation of effects into the life cycle components of selection would be crucial (McGraw and Antonovics 1983; Potvin 1986).

Since the early 1940s the importation of vegetable transplants from the southern United States has provided a

corridor for the direct immigration of southern genotypes of eastern black nightshade into northern agroecosystems. This method of entry into peripheral habitats was confirmed, especially for tomato imports from North Carolina (section 1.5). The ability of the North Carolina EBN seedlings to survive transplantation into the tomato crop, to flower and successfully produce viable seed in that first season, and of the seedlings to emerge the following year (section 1.6) reflect the potential for incorporation of southern genotypes into the gene pool of northern populations of EBN. The predicted inability of southern phenotypes to reach reproductive maturity under short crop cultural conditions may limit its invasive potential. But hybridization of NC with resident plants in the season of transplantation (when NC plants successfully flowered) is plausible, especially given the higher outcrossing rates of the NC plants. This hybridization would be expected to release variability in the F_2 generation in many of the life-history characters, such as those adult traits which showed minimal variability (e.g. age to first flowering), and the subsequent incorporation of southern genes into the agrestal population. While the similarity of the ruderal and agrestal populations in these adult traits (Chapter 5), and the absence of the diagnostic southern allele (Chapter 2) indicate few "Southern" NC genes have introgressed into the NA populations, this whole process is relatively recent and

may take a number of generations to build up a sufficient density to detect such increased variability. This release of variability would result in an increased potential to respond to the variable cultural conditions associated with crop rotation, or any other changes which may occur in agroecosystems. Currently the influx of individuals from Georgia appears to be less important (section 1.5), but the occurrence of the same PGM allele ("S") and the similarity of its floral morphology in these populations (Chapter 2) suggest a possible role in past immigration events. However, it is unlikely that the northern agrestal populations were founded by the slow PGM homozygote imported from Georgia, because both the slow homozygous families expressed the typical "southern" phenotype with long pre-reproductive period and high susceptibility to herbicides. Of course, these may have introgressed into an already founded population in a manner similar to that outlined above, with the resulting evolution of a shorter pre-reproductive period and herbicide resistance.

Few studies have dissected genetic and plastic components of variability over a range of environmental gradients relevant to agroecosystems, specifically contrasting ruderal and agrestal populations at each stage of the life cycle. This study also illustrates the utility of agrestal weeds in the study of colonization genetics. The well documented, readily available historical records,

the presence of identifiable biotic and abiotic selection agents, the availability of different cultural regimes to contrast potential response to selection, and the applied aspect of control make agrestal weeds ideal model systems to test a wide range of ecological and genetical hypotheses.

Appendix A

DETECTION OF AN ENZYME POLYMORPHISM

Extremely low levels of allozyme variation have been found in the majority of annual, self-pollinating weed species (see Chapter 2), to the extent that many populations are totally monomorphic. Given this low probability of detecting enzyme polymorphisms, a small scale, preliminary survey was undertaken to search for genetic markers. Genetic markers were sought in order to assess levels of population differentiation, and to measure the amount of outcrossing. Based on floral structure and phenology, the majority of weedy species, including EBN (Bassett and Munro 1985) are thought to be autogamous, but few studies have actually measured outcrossing rates (see Chapter 2).

Enzyme extracts were obtained by grinding seeds, roots and/or leaf tissue in 0.1 M Tris-HCl, pH=8.0 with B-mercaptoethanol (5 ul/10 ml buffer) and PVPP (Polyvinyl-polypyrrolidone) on ice. Initially the crude extract was screened using horizontal starch gel electrophoresis (Soltis et al. 1983) resulting in 10 resolvable enzyme systems (Table A.1). Subsequently I switched to cellulose acetate electrophoresis (Hebert and Beaton 1989), where 4 additional enzymes were resolved, for a total of 14 resolvable enzymes encoding for 20 loci (Kephart 1990; Table A.1). In total, 3-4 plants from each of 11 populations (6 counties), representing both ruderal and agrestal habitats were sampled

Table A.1 Details of the electrophoretic screening carried out on starch and cellulose acetate, and the number of loci detected for each enzyme

STARCH	CELLULOSE ACETATE	
Buffer Systems[1]		
Discontinuous tris-citrate Tris borate EDTA Histidine Lithium hydroxide	Tris glycine Tris maleate	
Enzymes		**# loci**
AAT (GOT) (Asparate aminotransferase)	AAT	2
G-6-PD (Glucose-6-phosphate DH*)	---	1
GDH (Glutamate DH)	---	1
IDH (Isocitrate DH)	IDH	1
PEP (Leucyclglycine peptidase)	PEP	1
PGI (GPI) Phosphoglucose isomerase	PGI	2
PGM (Phosphoglucomutase)	PGM	2
6-PGD (6-phosphogluconate DH)	---	2
SOD (TO) (Superoxide dismutase)	---	1
SKDH (Shikimic DH)	---	1
---	ADH (Alcohol DH)	1
---	AMY (Amylase)	1
---	FUM (Fumarase)	1
---	MDH (Malate DH)	3

* DH=dehydrogenase

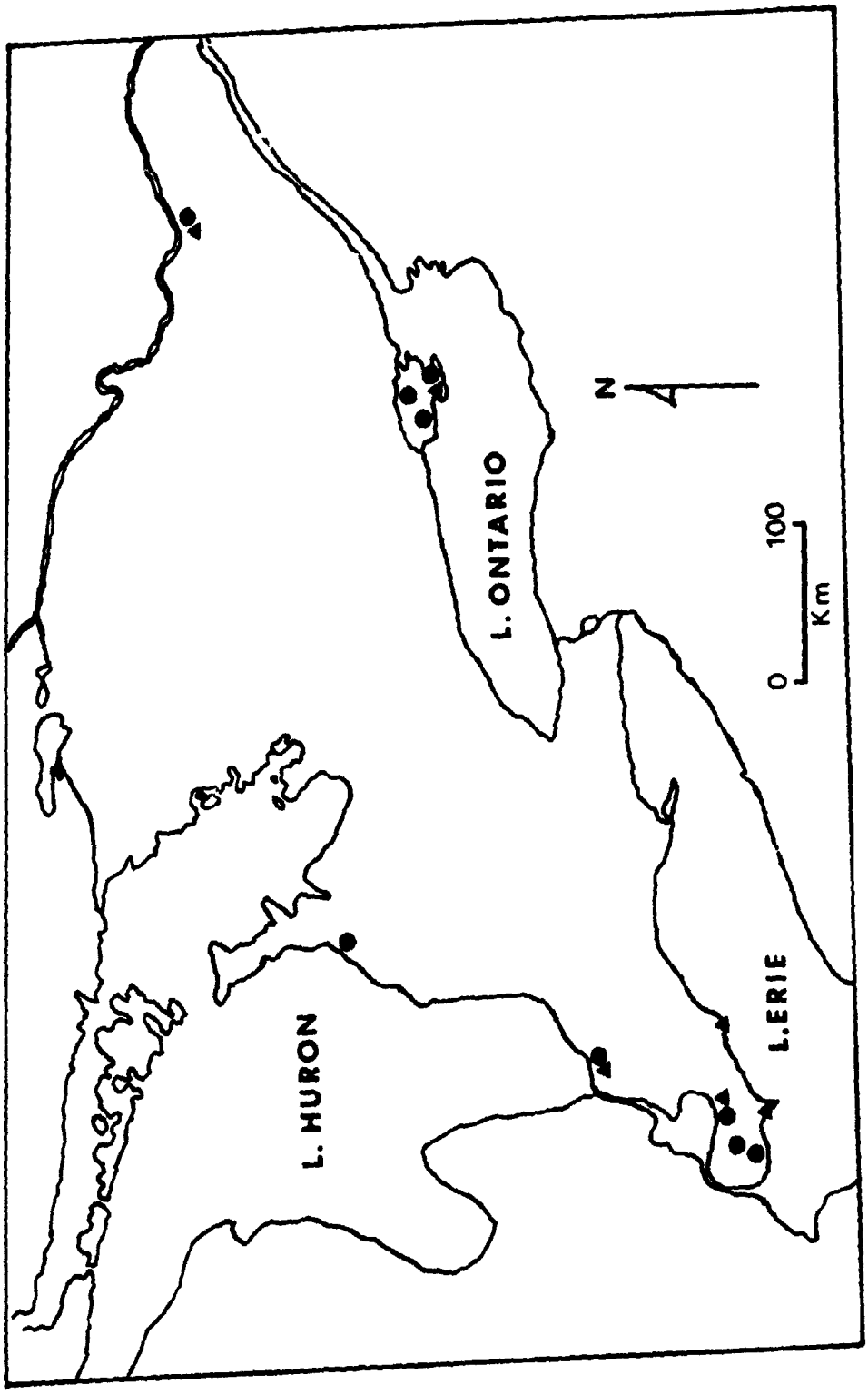
1 Soltis *et al*. 1983; Hebert and Beaton 1989

from across Ontario (Fig. A.1). Agrestal populations represented a variety of crops (tomato, soybeans and alfalfa). There was no evidence of enzyme multiplicity (i.e., "fixed" heterozygosity") at any of the loci, confirming that EBN is not a polyploid, which is similar to Warwick's (1990a) finding for Datura (see below).

All putative enzyme loci were monomorphic regardless of population origin. Warwick (1990a) found only a single genotype in her survey of Datura stramonium, a member of the Solanaceae and a weed with a very similar life history and historical background to EBN. This lack of variability is also consistent with Whalen's (1979) survey of eight weedy annual species of Solanum in the section Androceras. In his survey, all but one of the species were totally monomorphic. The single variable species was polymorphic at only one locus. Whalen suggested that historical factors (frequency and severity of genetic bottlenecks, and greater age of perennials) acccunted for lower genetic variation detected in weedy annuals compared to perennial desert species. He discounted the role of breeding system due to their similarity in annual and perennial species.

In the current study, further sampling within Ontario was not pursued, as this was not the focus of the thesis, and as was mentioned, other studies have shown that most populations of species with similar life histories contain single genotypes.

Fig. A.1 Populations of EBN sampled for the electrophoretic survey. ●:Populations screened on starch gels; ▲:populations screened on cellulose acetate gels.


L. ONTARIO
L. HURON
L. ERIE
N
0
100
Km

Plants sampled from two transplant farms in Georgia, and individuals discovered as contaminants in crates of tomato transplants originating from North Carolina (see section 1.6) were screened with the above samples. A polymorphism was detected at the phosphoglucomutase-2 (PGM) locus. All NC nightshade plants subsampled from those found as contaminants in 1988 and 1989 (n=153) were fixed for the alternate (fast) Pgm-2 allele, compared to the Ontario populations which are fixed for the slow allele. Populations sampled in GA contained both homozygotes. Of 8 GA individuals sampled, 6 were fast and 2 were slow homozygotes. The generation of heterozygotes upon crossing of the 2 different homozygotes (n=13), and the true breeding of parents raised in the greenhouse for 2 generations, confirms the genetic basis of this polymorphism. This polymorphism was subsequently used to establish field populations to estimate outcrossing rates (Chapter 2), and to evaluate the establishment of southern genotypes in Ontario agroecosystems (section 1.6).

Appendix B

IMPORTATION OF OTHER WEED SPECIES WITH TOMATO TRANSPLANTS (Chapter 1)

In addition to EBN, the weed species most often encountered in the transplant crates were lambsquarters (*Chenopodium album*), pigweed (*Amaranthus* spp.) and spurge (*Euphorbia esula*). Hairy nightshade (*Solanum sarrachoides*) has also been included in Table B.1, because of its close taxonomic relationship to EBN, and its current status as a rapidly expanding pest species in Ontario, which may soon displace EBN in tomato fields (Hermanutz and Weaver 1991). Sporadic occurrences (<5 crates) of lady's thumb (*Polygonum persicaria*), foxtail (*Setaria* spp.), barnyard grass (*Echinochloa crus-galli*) and wild buckwheat (*Polygonum convolvulus*) were detected.

In 1987, lambsquarters and spurge seeds were detected more often, and in greater numbers in crates imported from NC than GA, (Table B.1). In 1988, spurge seed occurred in high densities in GA crates, but not at all in NC (Table B.1). Probability of contamination was greater in NC crates for lambsquarters and pigweed, but infestation rates were lower than GA crates. A single hairy nightshade seed was detected in GA crate (Table B.1). Plants of all the species detected as seeds, were also found in these crates.

In 1989, the seed and plant burdens were much greater than in the two preceding years (Table B.1). Lambsquarters

Table B.1 Seed contamination of tomato transplants imported from Georgia (GA) and North Carolina (NC) by common weed species other than EBN, evaluated by proportion of crates contaminated (%) and mean # seeds/kg (S.D.). See Table 1.1 for # crates screened per sample

		Species[a]							
		LQ		PW		SP		HA	
DATE	SOURCE	%	$\bar{X}$ (SD)	%	$\bar{X}$	%	$\bar{X}$	%	$\bar{X}$
1987	GA	2.8	1.6	0	0	11.1	2.6 (1.43)	0	0
	NC	20.0	3.8	0	0	40.0	7.4 (7.76)	0	0
1988	GA	24.8	7.0 (8.24)	9.0	7.2 (8.01)	35.3	5.8 (9.04)	0.8	1.1
	NC	50.0	3.3 (1.83)	37.5	1.2 (0.06)	0	0	0	0
1989	GA	32.9	18.9 (36.85)	11.4	4.1 (3.37)	48.1	7.7 (13.22)	5.1	22.1 (29.17)
	NC	0	0	37.5	11.3 (5.41)	12.5	2.1 (0.67)	0	0

[a] LQ=lambsquarters (Chenopodium album)
PW=pigweed (Amaranthus spp.)
SP=spurge (Euphorbia esula)
HA=hairy nightshade (Solanum sarrachoides)

in GA crates, and pigweeds in NC crates were especially dense. Seedlings of these species were evident, but in much lower numbers than the EBN. With the exception of nutsedge (*Cyperus* spp.), which was often found with intact tubers and in numbers in excess of 100, these weed densities were less than 5 seedlings per crate. It is of interest that all four crates which were contaminated with hairy nightshade seed in relatively high densities, originated from the same grower.

These results suggest that in addition to EBN, populations of many of the weeds which are currently important economic pests in Ontario (Alex 1982) may have been initiated by, or supplemented with, southern genotypes. Successful establishment and reproduction of these imports in the north must be documented to assess potential colonization. Many of the current problems of control may be due to the additional variation introduced by southern immigrants.

Appendix C

AMONG POPULATION VARIABILITY IN SEED PRODUCTION (Chapter 2)

Fecundity (total life time seed production) differences among populations cannot be easily assessed in field populations of EBN due to its indeterminate habit, and the staggering number of berries produced. In lieu of fecundity estimates, the number of seeds/berry were assessed to compare population differences. See section 2.2.1 for sampling design. The number of viable seeds/berry were counted before the seeds were used in the estimate of outcrossing.

Regardless of method of fertilization (selfed or outcrossed), the mean number of propagules produced per berry is large for all populations, ranging from 70.8-88.5 (Table C.1). There was significant among population variation in the number of seeds set/berry, with the Georgia populations producing the greatest number of seeds/berry (Table C.1), and the NC population the least.

Lack of knowledge of the fecundity differences among populations, does not allow rejection of the this factor as a plausible explanation for the observed decrease in individual outcrossing rates in the northern marginal populations. However, as even the populations which set fewer seeds/berry produce large numbers of seeds, it seems unlikely that reproductive assurance would favour decreased levels of outcrossing in northern populations.

Table C.1 Among population variability of EBN in the mean (SE) number of viable seeds produced per berry. Probability values based on Krukal-Wallis test for population differences. N=number of berries; F=number of families

	Number of seeds/berry				
Population	N	F	Mean	SE	CV
GA	23	4	88.5	2.61	14.16
NC	16	2	70.8	3.00	16.97
H	16	2	84.8	4.19	19.77
W	16	2	77.2	3.86	18.03
P	16	2	76.4	2.61	13.66
R	5	1	82.4	4.15	11.27
			p=0.0019		

Appendix D

SEEDLING SIZE VARIABILITY AMONG GROUPS (Chapter 4)

As the degree of response to herbicide can be size dependent, I tested if the initial treatment allocations were random (i.e., plants among treatments were similar in size). Within each population, for each size measure, pre-application treatment differences were not significant (all Kruskal-Wallis $x^2<3.00$, $p>0.05$) with only three exceptions (Table D.1).

Variation in growth response to the herbicide may also be a consequence of pre-application size differences among populations, rather than due to genetic difference in tolerance. To investigate this possible bias, one measure of pre-application size (seedling height at 22 days) was added as a covariate (log height) in the ANOVA (PROC GLM) for each of the three dependent variables. In all cases the covariate was not significant, indicating any growth response variability was the result of genetic differences among populations in herbicide tolerance.

Table D.1 Pre-application size variability (mean ± SD) among treatments within each population of EBN, 22 days after sowing. Length/width measures are for 3rd leaf

TYPE/ Pop.	Trt[a]	Pre-application size measures			
		Height(cm)	#leaves	Length(cm)	Width(cm)
NR R	1	3.7 (0.28)	5.6 (0.71)	5.1 (0.52)	3.6 (0.39)
	2	3.7 (0.32)	5.8 (0.52)	5.3 (0.51)	3.7 (0.36)
	3	3.7 (0.28)	5.8 (0.62)	5.2 (0.45)	3.6 (0.33)
P	1	4.4 (0.72)	5.6 (0.51)[b]	4.8 (0.73)	3.3 (0.45)[b]
	2	4.5 (0.85)	5.8 (0.37)	5.1 (0.85)	3.5 (0.52)
	3	4.5 (0.86)	5.9 (0.53)	5.2 (0.64)	3.5 (0.34)
NA H	1	3.5 (0.28)	6.4 (0.67)	4.5 (0.54)	3.3 (0.36)
	2	3.4 (0.31)	6.4 (0.58)	4.7 (0.47)	3.4 (0.35)
	3	3.6 (0.30)	6.4 (0.53)	4.6 (0.45)	3.3 (0.33)
W	1	3.3 (0.37)	6.1 (0.44)	4.6 (0.50)	3.3 (0.36)
	2	3.7 (0.29)	6.2 (0.53)	4.8 (0.34)	3.5 (0.29)
	3	3.7 (0.29)	6.3 (0.44)	4.9 (0.40)	3.5 (0.25)
SA G1	1	3.3 (0.33)	5.2 (0.41)	5.1 (0.44)	3.5 (0.49)
	2	3.2 (0.24)	5.1 (0.35)	5.3 (0.49)	3.5 (0.39)
	3	3.1 (0.37)	5.1 (0.34)	5.1 (0.62)	3.5 (0.57)
G2	1	3.4 (0.36)	5.2 (0.44)	4.9 (0.62)	3.5 (0.59)
	2	3.5 (0.35)	5.4 (0.50)	5.1 (0.46)	3.6 (0.40)
	3	3.4 (0.32)	5.3 (.052)	5.1 (0.43)	3.6 (0.39)
NC	1	2.7 (0.30)	5.5 (0.51)	4.2 (0.43)[b]	3.2 (0.30)
	2	2.8 (0.38)	5.6 (0.51)	4.3 (0.40)	3.2 (0.28)
	3	2.8 (0.36)	5.6 (0.49)	4.5 (0.40)	3.3 (0.29)

[a] Sample sizes=25,25,40 for 3 trt except for G1: 15,15,24

[b] Significant among trt heterogeneity; 0.04>p<0.05. See text for explanation.

Appendix E

CORRELATION MATRIX OF GROWTH TRAITS (Chapter 5)

The suite of traits which was analyzed in Chapter 5 were chosen to minimize the correlation among them. Table E.1 is the half matrix of Spearman Rank Correlation Coefficients pooling populations (excluding NC) and treatments.

Table E.1 Correlation matrix (r_s) of all growth traits of EBN measured in the nutrient experiment (n=252). See Table 5.1 for trait acronyms. Entries in bold print designate significant ($p<0.05$) correlations.

	HT1	LA	DW	FLB	HT2	SPL2	FL1	BR	LV	AGE1	HT3	SPL3	INTER	BD	SIDE	ANG	BCL	BSET	RDW	RTDW	VDW	RPDW	HABIT
HT1	--	.70	.71	.59	-.08	-.13	-.04	.02	-.37	-.64	-.33	-.13	-.24	.11	.31	-.50	.18	.17	.32	-.58	.28	.72	.61
LA		--	.97	.21	.25	.19	.15	.39	.01	-.34	.04	.20	.19	.36	.38	-.33	.29	.19	.06	-.45	.44	.60	.39
DW			--	.22	.25	.20	.14	.36	.01	-.35	.04	.20	.19	.34	.36	-.33	.30	.22	.08	-.45	.44	.61	.39
FLB				--	-.62	-.67	-.12	-.30	-.75	-.80	-.64	-.68	-.68	-.24	.05	-.45	.02	-.07	.53	-.42	-.12	.55	.60
HT2					--	.95	.16	.40	.70	.64	.78	.95	.89	.60	-.38	.23	.21	.21	-.64	.05	.54	-.19	-.31
SPL2						--	.16	.44	.79	.71	.75	.98	.85	.47	.23	.33	.19	.22	-.58	.17	.42	-.28	.43
FL1							--	.13	.05	.06	.19	.16	.20	.20	.12	.11	.54	-.03	-.12	-.01	.16	.06	.07
BR								--	.51	.22	.44	.44	.48	.53	.29	-.13	.23	.23	-.26	-.19	.46	.14	.00
LV									--	.77	.60	.78	.75	.20	-.08	.39	.06	.22	-.43	.36	.14	-.41	-.55
AGE1										--	.67	.69	.68	.16	-.13	.64	-.01	.08	-.55	.57	.03	-.73	-.73
HT3											--	.76	.78	.58	.27	.38	.15	.08	-.61	.23	.44	-.30	-.52
SPL3												--	.89	.52	.29	.29	.17	.20	-.64	.12	.47	-.28	-.39
INTER													--	.59	.35	.28	.18	.14	-.68	.12	.51	-.26	-.33
BD														--	.79	-.26	.21	.03	-.71	-.49	.92	.14	.27
SIDE															--	-.43	.16	.00	-.54	-.68	.80	.30	.59
ANG																--	.02	.00	-.09	.78	-.37	-.58	-.78
BCL																	--	.42	.08	-.09	.18	.32	.05
BSET																		--	.17	.03	.02	.19	-.03
RDW																			--	.16	-.64	.49	.12
RTDW																				--	-.63	-.56	-.82
VDW																					--	.27	.39
RPDW																						--	.66

Appendix F (Chapter 5).

Table F.1 Correlation, importance and significance of the canonical functions used to discriminate types in the canonical discriminant analysis. The likelihood ratio measures the amount of discrimination of the functions. All F approximations associated with likelihood values of the first 5 functions were significant, and the remaining three functions generated were not

	Canonical Correlation	Eigenvalue	Proportion of variation	Likelihood Ratio
CAN 1	0.991	53.58	0.606	0.0000087
CAN2	0.979	22.53	0.255	0.0004767
CAN3	0.937	7.25	0.082	0.011218
CAN4	0.897	4.11	0.047	0.092497
CAN5	0.620	0.62	0.007	0.472744

Table F.2 Total structure correlations between the original variables and the canonical functions

	FUNCTION			
VARIABLE	CAN1	CAN2	CAN3	CAN4
RTDW	-0.963*	0.136	-0.055	-0.013
SIDE	0.954*	0.126	-0.212	0.074
VDW	0.901*	0.311	-0.087	-0.243
BD	0.807	0.556	-0.026	-0.113
HT3	0.234	0.935*	0.089	0.116
LV	-0.194	0.933*	-0.220	0.039
AGE1	-0.368	0.877*	-0.245	-0.054
RDW	-0.411	-0.628	0.545*	0.245
RPDW	0.602	-0.525	-0.168	0.408
BR	0.449	0.730	0.331	0.139

* Variables used to name the function

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