

ECOTYPIC DIFFERENTIATION AND PLANT GROWTH IN THE LUQUILLO MOUNTAINS OF PUERTO RICO

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MYSTER, R. W. & FETCHER, N. 2005. Ecotypic differentiation and plant growth in the Luquillo Mountains of Puerto Rico. A greenhouse experiment was performed to investigate ecotypic differentiation among tropical trees along an elevation gradient in Puerto Rico, USA, using *Clibadium erosum* cuttings and *Prestoea acuminata* var. *montana* seedlings. *Clibadium erosum* cuttings (1) grew more after nitrogen (N) addition and after phosphorus (P) addition but there was no N+P effect, (2) were larger from El Verde (elevation 350 m) compared with Pico del Este (1000 m), (3) showed increased photosynthesis after N addition, and (4) from Pico del Este were higher in photosynthesis during one sample period but lower in the other. We also found that *P. acuminata* var. *montana* seedlings (1) had greater growth after N addition, (2) had different leaf parameters after N addition, and (3) showed an increase in photosynthesis after N addition. We conclude that because only three out of ten plant traits showed differentiation, ecotypes for these two tree species on this neotropical mountain range may not exist.

Key words: *Clibadium erosum* - *Prestoea acuminata* var. *montana* - nitrogen - phosphorus - population origin

MYSTER, R. W. & FETCHER, N. 2005. Pembezaan ekotip dan pertumbuhan tanaman di Pergunungan Luquillo, Puerto Rico. Satu eksperimen rumah kaca dijalankan untuk menyiasat pembezaan ekotip di kalangan pokok tropika sepanjang kecerunan ketinggian di Puerto Rico, Amerika Syarikat, menggunakan keratan *Clibadium erosum* dan anak benih *Prestoea acuminata* var. *montana*. Keratan *C. erosum* (1) tumbuh lebih pesat selepas pertambahan nitrogen (N) dan pertambahan fosforus (P) tetapi pertambahan N + P tiada membawa kesan, (2) dari El Verde lebih besar (ketinggian 350 m) berbanding Pico del Este (1000 m), (3) menunjukkan peningkatan fotosintesis selepas pertambahan N, dan (4) dari Pico del Este lebih tinggi dalam fotosintesis pada satu tempoh sampel tetapi lebih rendah pada tempoh yang satu lagi. Kami juga mendapati anak benih *P. acuminata* var. *montana* (1) tumbuh lebih pesat selepas pertambahan N, (2) mempunyai parameter daun yang berbeza selepas pertambahan N, dan (3) menunjukkan peningkatan fotosintesis selepas pertambahan N. Kami membuat kesimpulan bahawa oleh sebab hanya tiga daripada 10 ciri pokok menunjukkan pembezaan, ekotip kedua-dua spesies di atas banjaran gunung neotropik ini mungkin tidak wujud.

Introduction

Species distributions correlate with gradients of temperature and rainfall on a variety of spatial scales (Walter 1979, Myster 2001). Within these distributions, species may have their greatest densities in the middle of gradients and thin out at the edges (Austin *et al.* 1994, Myster *et al.* 1997), becoming ecotypes or locally adapted populations (well documented for plant species from the temperate, boreal, and Arctic zones; see Hufford & Mazer 2003 and references therein). Whether grown in a common environment or in a reciprocal transplant design, ecotypes typically exhibit marked differences in growth and physiology that presumably reflect selection for increased fitness in their native environment (McGraw & Antonovics 1983, Bennington & McGraw 1995).

Past studies have shown that species that evolve in sites with stressful climates or low nutrients can have reduced productivity compared with species or subspecies from more favourable environments (Grime 1979). Furthermore, ecotypes from cooler environments such as alpine or northern tundra tend to have smaller stature than ecotypes from warmer environments when all are grown in a common garden (Shaver *et al.* 1986, Fetcher & Shaver 1990). In general, ecotypes from cooler habitats tend to be less plastic than those from warmer habitats (McGraw & Antonovics 1983, Fetcher & Shaver 1990).

The elevation gradient in the Luquillo Mountains, Puerto Rico, USA, is accompanied by declines in species diversity, mean temperature, and solar radiation, and by an increase in rainfall (Brown *et al.* 1983). Lower temperatures and wetter soils at high elevation may make nutrients less available because of reduced decomposition (Cavelier 1996). Thus if ecotypic differentiation occurred, populations from higher elevations might have adapted to conditions of lowered availability and exhibit less plastic responses to nutrient addition. To test this idea, we designed a greenhouse experiment to examine the response of populations from higher and lower elevations in the Luquillo Experimental Forest (LEF) to nutrient addition. We used two species, a pioneer shrub (*Clibadium erosum*) and a palm (*Prestoea acuminata* var. *montana*), in this experiment and addressed the following questions:

- (1) Do individuals from populations of *C. erosum* and *P. acuminata* var. *montana* located at 350 m and 1000 m grow similarly in a common controlled environment (greenhouse)? and
- (2) Do the populations respond differently to the addition of the nutrients N and P?

Materials and methods

In the spring of 1991 we collected 48 tree cuttings close to 10 cm in height from a small number of genotypes of *C. erosum* and 48 tree seedlings of *P. acuminata* var. *montana* at each of two sites in the Luquillo Experimental Forest (LEF), northeast

Puerto Rico, USA (18° 20' N, 65° 49' W). The sites are located at El Verde (EV: 350 m elevation and 3460 mm annual precipitation) and Pico del Este (PE: 1000 m elevation and 4200 mm annual precipitation). The sites are 9 km apart and there is a reduction in both mean maximum temperature (7 °C less) and solar radiation (50% less) with an increase in elevation. We planted the seedlings and cuttings in one-gallon pots until they were well established, and then transplanted them into five-gallon pots in a greenhouse at the Agricultural Experimental Station, University of Puerto Rico, Rio Piedras. The 192 pots (2 species x 2 sites x 2 treatments x 2 levels of each treatment x 2 harvests x 6 replicates) were filled with a 1:1:2 mix of river sand, potting mix (SOGEMIX, Sungro Horticulture Corp., Bellevue, WA, USA), and commercial topsoil that was homogenized with a soil mixer. Both study species occur throughout the entire forest gradient from tabonuco (*Dacryodes excelsa*) forest at lower elevations to elfin forest at higher elevations (Myser *et al.* 1997).

A factorial randomized block design with two treatments, two levels of each treatment and six replications was used to assess ecotypic differences in *C. erosum* and *P. acuminata* var. *montana*. For the phosphorus (P) treatment, the amount for naturally occurring P in the potting mixture was measured following extraction by IST-EI solution (Hunter 1974) and this became the low P treatment of 0.307 g m⁻². The high P treatment was to double the low P treatment using triple superphosphate, which was mixed with the soil at the beginning of the experiment and added at six-month intervals directly to the roots at a rate of 0.614 g m⁻². The amount of naturally occurring nitrogen (N) in the potting mixture was measured using the salicylic acid-thiosulfate modification of the Kjeldahl method (Black 1965) and this was the low N treatment. The high N treatment was twice the low N treatment; the nitrogen source was urea and ammonium nitrate added monthly at the rate of 0.095 g m⁻². Micronutrients at the rate of 0.13 g m⁻² was also added monthly. Plants were watered by a misting system controlled by an evaporative switch that turned on the water at roughly 20-min intervals. Maximum and minimum air temperatures were monitored in the greenhouse from December 1991 to October 1992 and averaged 35.8 and 22.2 °C respectively.

Each species was harvested twice; one individual from each combination of treatment and block was taken at each harvest. *Clibadium erosum* was harvested in July 1991 and May 1992, whereas *P. acuminata* var. *montana*, which had slower growth rates, was harvested in March and October 1992. After each harvest, all leaves (expanded and non-expanded), all branches and stems, and all primary and secondary roots of each individual were dried for more than 48 hours at 60–65 °C and weighed. Roots were washed carefully using tap water and then separated into primary and secondary roots. For the monocot *P. acuminata* var. *montana* (which has a diffuse root system), roots projecting from the stem base were considered primary. Foliar area of expanded and non-expanded leaves was measured with a leaf area meter (LI-3100, LiCor, Inc., Lincoln, Nebraska, USA). Foliar nitrogen concentration was measured with a Carlo-Erba CHN analyser. To determine the pattern of biomass allocation, we calculated leaf area ratio (LAR, foliar area/total mass), leaf mass ratio (LMR, foliar mass/total mass) and specific leaf mass (SLM, foliar mass/foliar area) for each plant.

Instantaneous gas exchange measurements were made on healthy, recently expanded, mature leaves of *C. erosum* in July 1991 and January 1992 and of *P. acuminata* var. *montana* in February 1992. A portable photosynthesis system (LI-6250, LiCor, Inc., Nebraska, USA) in closed mode was used to measure maximum CO_2 assimilation rate (A_{max}), stomatal conductance to water vapour (g), internal CO_2 partial pressure (P_i), and instantaneous water use efficiency (WUE). To ensure complete induction of photosynthesis, measurements were made in late morning and midday outside the greenhouse after more than 5 min of direct exposure to the sun. Total measurement times greater than 60 s were avoided to prevent elevated leaf temperatures. The experiment had a randomized block 3-way factorial design with N addition, P addition, and population origin as factors. Biomass data were log-transformed to stabilize variance. Because of missing data due to plant mortality, we used Type III sums of squares calculated by SAS-PC's GLM program (SAS 1985).

Results

Biomass of *C. erosum* measured in July 1991 was significantly greater in response to N and N+P fertilization ($F = 35.74$; $p < 0.0001$) compared with controls at both sites. Further, there was no additional benefit of adding P because biomass in the N+P treatment was the same as that of the N treatment alone. There was no site effect on biomass in this harvest. Addition of N lowered leaf area ($F = 6.5$; $p < 0.01$), but there was no significant treatment effect on leaf mass ratio or specific leaf mass. At the second harvest (May 1992), biomass of *C. erosum* increased in response to N addition, P addition and N+P addition at similar levels ($F = 4.4$; $p < 0.05$). Furthermore, there was significant difference in biomass between the two populations with the EV population being greater than the PE population (1.3 g mean vs. 1.0 g mean; $F = 4.2$; $p < 0.05$). At this harvest, there were no significant effects on biomass allocation or foliar nitrogen concentration.

Addition of N significantly increased *C. erosum* A_{max} relative to controls measured in July 1991 (13.1 vs. 9.2; $F = 7.6$; $p < 0.01$). Unfertilized plants from the PE population had higher values for A_{max} than did the EV population (12.2 vs. 9.3; $F = 4.2$; $p < 0.05$). WUE was higher with added N ($F = 4.6$; $p < 0.05$), but stomatal conductance and internal CO_2 were unaffected. In January 1992, N addition again significantly increased A_{max} (14.2 vs. 9.7; $F = 8.7$; $p < 0.0001$). There was also a significant 3-way interaction between N addition, P addition and population origin ($F = 5.2$; $p < 0.05$) due to higher A_{max} for the El Verde population with the addition of N+P than for the Pico del Este population with the same treatment. Water use efficiency, stomatal conductance and internal CO_2 pressure were unaffected by treatment or population origin.

In the harvest of March 1992, biomass of *P. acuminata* var. *montana* increased in response to N addition ($F = 16.17$; $p = 0.0005$). This also happened in October 1992 ($F = 7.2$; $p < 0.01$). Foliar N was increased significantly ($F = 13.4$; $p < .01$) by N addition in March, but not in October. Addition of N decreased leaf area in March ($F = 8.7$; $p < 0.01$) and in October ($F = 5.6$; $p < 0.02$). Nitrogen fertilization resulted in higher specific leaf mass in March ($F = 4.5$; $p < 0.05$) but not in October. When

measured in March, population origin had a significant effect on leaf area ratio ($F = 13.6$; $p < 0.001$), with the PE population having the higher allocation to roots.

A_{\max} of *P. acuminata* var. *montana* was significantly increased by N addition (5.5 vs. 3.9[control]; $F = 7.9$; $p < 0.001$) as was WUE ($F = 4.7$; $p < 0.05$). Stomatal conductance and P_i were unaffected. There was no effect of population origin on photosynthetic parameters.

Discussion

In general, there was little evidence for ecotype differentiation in either species studied because only three (biomass, leaf area ratio, A_{\max}) out of ten measured traits (biomass, foliar area, foliar N, LAR, LMR, SLM, A_{\max} , g, P_i , WUE) showed a significant effect of population origin. Whether the differences that we did find represent adaptation to local environmental conditions is open to question. However, we found more differences between populations in the present study than in the companion field study (Fetcher *et al.* 2000). This may have been the result of the daytime temperatures observed in the greenhouse, which were higher than those encountered in the Luquillo Mountains (Brown *et al.* 1983) and which could have accentuated any physiological differences between the populations. Alternatively the field gradient may not be extreme enough to select for ecotypes (Fetcher *et al.* 2000).

If such differences exist, the populations from El Verde (annual mean maximum and minimum temperatures 26.0 °C and 21.5 °C) would be more likely to resist the negative effects of high temperature than the populations from Pico del Este (annual mean maximum and minimum temperatures 19.2 °C and 17.8 °C; Brown *et al.* 1983). The greater biomass for the EV population of *C. erosum* at the second harvest would be consistent with this interpretation as would be the observation of higher A_{\max} in the N+P treatment measured in January 1992. The observation of lower photosynthetic rate for the EV population of *C. erosum* measured in July 1991 is not consistent, however. Another factor that calls the adaptive value of the observed difference between populations into question is that the differences between populations were not consistent across harvests.

In the present study, the most significant effects were due either to N addition or to the interaction between N and P, which suggests that N could limit growth of these species under field conditions. A_{\max} increased with N addition, a result that is consistent with most studies. In terms of percentage change, biomass and A_{\max} of *C. erosum* were about as responsive to added N as were those of *P. acuminata* var. *montana*. These results contrast with those of the companion field experiment (Fetcher *et al.* 2000). Individuals of *C. erosum* grown at El Verde had more than 10 times as much leaf biomass as did individuals grown at Pico del Este regardless of population origin, whereas plants of *P. acuminata* var. *montana* were of equal size at both sites (Fetcher *et al.* 2000). *Clibadium erosum* seems to respond more to amelioration of climatic regime, whereas *P. acuminata* var. *montana* appears to be more affected by the addition of N.

Finally, the most likely explanation for the lack of population differentiation in these species is high gene flow, which could overwhelm local selective pressures (Carronero 1996). Large-scale disturbances such as those caused by hurricanes (Weaver *et al.* 1986) could contribute to high gene flow by providing a multitude of suitable sites for establishment. Both *C. erosum* and *P. acuminata* var. *montana* can be considered successional species that establish themselves after disturbances and may be dispersed more widely than species that appear later in succession. *Clibadium erosum* is found in treefall gaps in Costa Rican montane forests similar to the LEF (Lawton & Putz 1988) and landslides in the LEF (Myster & Walker 1997). Although *P. acuminata* var. *montana* is shade tolerant (Lugo 1970), its recruitment is linked to large-scale disturbances by hurricanes (Weaver *et al.* 1986).

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References

- AUSTIN, M. P., NICHOLLS, A. O., DOHERTY, M. D., & MEYERS, J. A. 1994. Determining species response functions to an environmental gradient by means of a *B*-function. *Journal of Vegetation Science* 5:215–228.
- BENNINGTON, C. C. & MCCRAW, J. B. 1995. Natural selection and ecotypic differentiation in *Impatiens pallida*. *Ecological Monographs* 65: 303–323.
- BLACK, C. A. 1965. *Methods of Soil Analysis*. Agronomy 9, Part 2. American Society of Agronomy, and Soil Science Society of America, Madison, Wisconsin, U.S.A.
- BROWN, S. B., LUGO A. E., SILANDER, S. & LIEGEL, L. 1983. *Research History and Opportunities in the Luquillo Experimental Forest*. U. S. Forest Service General Technical Report SO-44. Institute of Tropical Forestry, Rio Piedras, Puerto Rico.
- CARRONERO, W. 1996. Genetic Structure and Gene Flow Rates along an Elevational Transect for the Tropical Palm *Prestoea montana*. M.S. thesis, University of Puerto Rico, Rio Piedras, Puerto Rico.
- CAVELIER, J. 1996. Environmental factors and ecophysiological processes along altitudinal gradients in wet tropical mountains. Pp. 399–439 in Mulkey, S. S., Chazdon, R. L. P. & Smith, P. (Eds.) *Tropical Forest Plant Ecophysiology*. Chapman and Hall, New York.
- FETCHER, N., CORDERO, R. A. & VOLTZOW, J. 2000. Lack of ecotypic differentiation: plant response to elevation, population origin and wind in the Luquillo Mountains, Puerto Rico. *Biotropica* 32: 225–234.
- FETCHER, N. & SHAVER, G. R. 1990. Environmental sensitivity of ecotypes as a potential influence on primary productivity. *American Naturalist* 136: 126–131.
- GRIME, J. P. 1979. *Plant Strategies and Vegetation Processes*. John Wiley & Sons, New York.
- HUFFORD, K. M. & MAZER, S. J. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology and Evolution* 18:147–155.
- HUNTER, A. H. 1974. *International Soil Fertility Evaluation and Improvement: Laboratory Procedures*. Department of Soil Science, North Carolina State University, Raleigh, North Carolina.

- LAWTON, R. O. & PUTZ, F. E. 1988. Natural disturbances and gap-phase regeneration in a wind exposed tropical cloud forest. *Ecology* 69: 764–777.
- LUGO, A. E. 1970. Photosynthetic studies on four species of rain forest seedlings. Pp. 81–102 in Odum, H. T. & Pigeon, R. F. (Eds.) *A Tropical Rainforest*. Office of Information Services, U.S. Atomic Energy Commission.
- MCCRAW, J. B. & ANTONOVICS, J. 1983. Experimental ecology of *Dryas octapetala* ecotypes I. Ecotypic differentiation and life-cycle stages of selection. *Journal of Ecology* 71:879–879.
- MYSTER, R. W. 2001. Mechanisms of plant response to gradients and after disturbances. *The Botanical Review* 67: 441–452.
- MYSTER, R. W., THOMLINSON, J. R. & LARSEN, M. C. 1997. Predicting landslide vegetation in patches on landscape gradients in Puerto Rico. *Landscape Ecology* 12: 299–307.
- MYSTER, R. W. & WALKER, L. R. 1997. Plant successional pathways on Puerto Rican landslides. *Journal of Tropical Ecology* 13: 165–173.
- SAS. 1985. *Users Guide: Statistics*. Version 5 edition. SAS Institute Inc. Cary, North Carolina.
- SHAVER, G. R., FETCHER, N. & CHAPIN III, F. S. 1986. Growth and flowering in *Eriophorum vaginatum*: annual and latitudinal variations. *Ecology* 67:1524–1535.
- WALTER, H. 1979. *Vegetation of the Earth and Ecological Systems of the Geo-biosphere*. Springer Verlag, New York.
- WEAVER, P. L., MEDINA, E., POOL, D., DUGGER, K., GONZALES-LIBOY, J. & CUEVAS, E. 1986. Ecological observations in the dwarf cloud forest of the Luquillo Mountains in Puerto Rico. *Biotropica* 18:79–85.