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Ecological correlates of seed mass variation in *Phoradendron juniperinum*, a xylem-tapping mistletoe

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Summary. We investigated several ecological correlates of seed mass variation in the hemiparasitic, xylem-tapping mistletoe, *Phoradendron juniperinum*. Mean seed mass varied two-fold among plants between the ages of 4 and 14 years old and was positively correlated with parental plant age. Both the standard deviation and the coefficient of variation in mean seed mass decreased with increasing plant age demonstrating that, on average, younger plants produced seed with more variable mass. Nitrogen concentrations (mg nitrogen per gram of seed) of both the seed and “fruit” (pericarp) were not correlated with mass or the age of the parent plant from which the seed was taken. However, the nitrogen content per seed (mg nitrogen per seed) was positively correlated with the mean seed dry mass and the age of the seed parent, suggesting that the carbon to nitrogen ratio of individual seeds remained relatively constant as seed mass increased and plants grew older. Seed germination ranged between 20% and 86% and was positively correlated with mass and parental plant age. Heavier seeds (seeds from older plants) also had the highest root radicle growth rates. Furthermore, the final root radicle length after 76 d of growth was positively correlated with seed dry mass. When grown on a medium containing an extract prepared from the host plant foliage, all seeds showed lower germination, grew more slowly and had shorter overall root radicles, but had significantly greater development of the haustorial “disks” (the holdfast which forms the host-parasite junction in *Phoradendron*) than seeds grown on a control medium. Our results suggest that, on average, seeds of greater mass produced by older plants have a greater total resource pool per propagule (fruit + seed). This resource pool may be important in conferring a greater potential for dispersal (fruit), survival, colonization, and establishment.

Key words: Mistletoe – Seed mass variation – Establishment – Carbon to nitrogen ratio – *Phoradendron juniperinum*

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Studies of variation in seed mass or size have led to the notion that seedlings from heavier or larger seeds may have a performance advantage in terms of higher growth rates and hence exhibit greater survivorship, establishment, and/or resilience to competition from neighbors (Black 1958; Schaal 1980; Mazer 1989) or other types of abiotic stresses (Harper et al. 1970; Salisbury 1974; Schimpf 1977; Galen et al. 1985; Wulff 1986; Wolf et al. 1986; Stanton et al. 1987). Although, on average, a larger seed may confer an ecological advantage for an establishing seedling in a “stressful” environment, variation in seed mass or size within a plant, plant population, or species may also confer an additional evolutionary advantage in environments that are both temporally and spatially variable. This “adaptive coin-flipping” or “bet-hedging” strategy may increase the probability that a fraction of the seed population persists through environmental situations that are of high risk, and may be one reason for the maintenance of variation in seed size, mass, or number. However, current bet-hedging models find that the maintenance of variation in seed mass or size is hard to achieve (Smith and Fretwell 1974; Kaplan and Cooper 1984; McGinley et al. 1987; Philippini and Seger 1989). Although several studies have demonstrated positive correlations between seed mass and establishment or survivorship, the basis of the correlation is poorly understood. That is, it is unclear whether seedlings emerging from larger seeds have improved performance characteristics (e.g. higher growth rates) that enhance survival or because a larger seed represents a greater resource pool which enhances a seedling’s ability to withstand longer intervals of high environmental stress levied during the establishment phase. In point of fact, both these reasons for the maintenance of larger seeds may occur simultaneously.

Interspecific variation in seed mass or size is correlated with an extremely broad array of ecological, physiological, taxonomic, and life history attributes in plants (Baker 1972; Fenner 1985; McGinley et al. 1987; Mazer 1989). At the intraspecific level, a potentially important correlate of seed mass variation that has been largely neglected is age of the parental plant, especially in non-arborescent, long-lived perennials (but see Cavers

and Steel 1984). Since there are few accurate methods for aging non-arborescent perennial plants, it is not surprising that most previous studies have focused on short-lived plants such as annuals and biennials. Here we report on variation in seed mass and seed nitrogen in the parasitic higher plant, *Phoradendron juniperinum*, a xylem-tapping mistletoe. We document correlations between seed mass with the age of the parent, growth, and establishment characteristics under a variety of conditions.

Seeds in this mistletoe species are either defecated or regurgitated on the host branches and foliage by birds. Establishment in all epiphytic parasites requires that the germinating seedling find a suitable penetration point in the host foliage or twig bark, establish a holdfast, and then successfully grow with the host tissues to form a parasitic union. During this initial establishment phase, the majority (if not all) of the resources for growth and establishment must be contained within the seed, since autotrophic activity is minimal.

Materials and methods

Phoradendron juniperinum Gray is the most common mistletoe found parasitizing *Juniperus osteosperma* (Torr.) Little, the Utah juniper, throughout the intermountain west of the United States. Like other members of the Viscaceae, *P. juniperinum* lacks ovules and thus the terms "seed" or "fruit", as used here, are not correct in the strict sense (Gill and Hawksworth 1961). However, as in previous studies of mistletoe seed biology (Lamont 1983; Sallé 1983), we adopt these terms as a matter of convenience and because these dispersal structures are ecologically analogous to true fruits and seeds in other angiosperms.

Fruits are one-seeded with a thick pericarp consisting of an epicarp, mesocarp, and endocarp (Sallé 1983). The mesocarp is composed of a sticky mucilaginous viscin that firmly adheres the seed to a surface. Although it has been reported that many parasitic plants have minute seeds (Fenner 1985), those of *P. juniperinum* are large and chlorophyllous with a single large embryo and massive endosperm.

Seed used in our studies were collected in the fall of 1987 and 1988 in a study population located in the southwestern corner of Utah, adjacent to the Coral Pink Sand Dunes State Reserve (37° 3' N, 112° 45' W, elevation 1855 m). The fruits were collected from 87 female plants (50 in 1987, 37 in 1988) of *P. juniperinum* that were parasitizing mature *J. osteosperma* of different sizes. Entire mistletoe plants were removed from their host, aged using a morphological index (Dawson et al. 1990 a), sealed in plastic bags to prevent desiccation, and transported back to the laboratory. In the laboratory, all fruit were removed from 50 of the 87 plants (35 in 1987, 15 in 1988) and sorted into one of three diameter size classes (<3, 3–6, and >6 mm). From a subset of 50 seeds, fresh weight of individual seeds were measured. The fruit (pericarp) was then removed from each seed by squeezing out the seed. Seeds were then placed on moist paper towels to dissolve the viscin layer surrounding the seed (Calvin 1966). After 24–36 hr, seeds were weighed to obtain seed fresh mass. Seeds were then dried at 70° C in a forced-draft oven for one week and then reweighed to obtain a seed dry mass. The "fruit" tissue surrounding each seed was dried for 48 hr before analyses.

Kjeldahl nitrogen determinations were made on dried fruit and seed with an autoanalyzer (Scientific Instruments Corp., model CFA200, Pleasantville, N.Y., USA) following the procedures outlined in Schulze and Ehleringer (1984). The Kjeldahl nitrogen analyses were conducted in two ways. First, seeds of the same mass on each of 40 female plants of different ages were combined and

ground to 40 mesh. Nitrogen concentration (mg N per g seed dry mass) was determined on seven replicate samples from each plant (n = 280). Second, individual seeds of known mass were analyzed (= nitrogen per seed; n = 5 per category for the same 40 parental plants).

Germination and growth studies

Seed from 37 plants (15 in 1987, 22 in 1988) were used in seed germination and root radicle growth experiments. Seeds were removed from the pericarp and the viscin layer dissolved as described above. After 24 to 36 h, all seeds were washed for 5 min in a dilute solution of sodium hypochlorite (approx. 1%) to kill any fungal pathogens on the seeds. Germination did not require after-ripening or scarification. Like seeds of other mistletoes, viability is short and clearly influenced by storage techniques, temperature, and light (unpubl. data; see also Lamont 1983; Beckman and Roth 1968). For the germination studies, seed transfers were made in a laminar-flow hood into petri dishes containing a medium of 1.5% gum agar (Sigma Chemical Co., St. Louis, MO. USA), 1% sucrose, and 1/4 strength Hoagland's solution (Epstein 1972; hereafter called control medium). Seeds of different mass were randomly chosen and placed 10 to a dish. The dishes were placed in an environmentally controlled growth chamber (27/20° C day/night, 14L/10D photoperiod, photon flux density of 0.5–0.85 mol m⁻² s⁻¹). Germinating seeds were checked every three days to assess germination percentages. A total of 9,680 seeds were tested.

After seeds had begun to germinate (9 to 15 days), the emerging root radicle length was measured on sixty seeds of variable initial mass and from seed parents of different ages (ten seeds in each of ten age classes). For these measurements, petri dishes were removed from the growth chamber, and without removing the lids on each dish, the root radicle length was determined with the use of an ocular micrometer on a dissection microscope (Wild, model M38, Heerburgg, Switzerland). The length was measured from the "collar" of the seed where the radicle emerged (see Fig. 8) to its tip. These studies were conducted both years, and the combined results are presented here.

Foliage extract studies

In 1988, two additional experiments were conducted to learn how seed germination and root radicle growth were affected by an extract of the host plant foliage in the growth medium. In the first experiment, seeds were either germinated 1) as before on the control medium or 2) on a treatment medium in which control medium was supplemented with a 1% juniper-leaf extract. Seed germination for both the control and treatment media (n = 56 paired trials, 10 seeds each) was determined as before, but in addition each seed was monitored for formation of a "disk" (holdfast) which develops at the distal end of the root radicle (n = 28 paired trials, 10 seeds each). The "disk" has previously been shown to be the primary organ of attachment between the parasite and its host (Kuijt 1969). In a second experiment, seed of different mass (obtained from 6, 9, 11 and 14 year old parents) were germinated on control medium. After 25 days, these seedlings were transferred to either a new dish of the control media or to the treatment media. Root radicle length was monitored as before both prior to and after transfer to different growth media. Sample sizes were a total of 75 seeds of different mass or 10 seeds in each of the four age categories depending on the specific experiment (given in more detail in the results section).

Scanning electron microscopy

Scanning electron micrographs of germinating mistletoe seeds were obtained to document whether different stages of development or different growth media affected differentiation or morphology. Ger-

minated seeds were fixed in 1% aqueous osmium tetroxide at 4°C in the dark for a minimum of 4 h. They were then coated with additional osmium by the OTO procedure of Kelly et al. (1973) and dehydrated in a graded series of ethanol solutions. The seeds were dried either by the critical point method from CO₂, or by sublimation of the fluorocarbon compound, Peldri II (Ted Pella, Inc., Redding, CA, USA) following the procedure outlined in Kennedy et al. (1989). Dried seeds were mounted on specimen stubs, sputter coated with gold, and examined in a Hitachi S-450 scanning electron microscope.

Data analysis

Correlational analyses were used in all experiments. Data were checked for normality and those that were not normally distributed were log transformed before the regression analyses were performed. Percent germination data were arcsine square root transformed, but the data are graphically presented as percent for clarity. In addition, univariate ANOVA's were used to examine the effects of seed mass or age of the seed parent (as the independent variables) on other variables. Multivariate ANOVA's (ANCOVA) were used to control for variation across years, variation in mass among seeds within an age class, and among individual seeds (mass) within an individual. Though mean seed mass and parental plant age were highly correlated (see below), seed mass was considered the key independent variable used in all the correlational analyses. For each analysis, the ANOVA results described the individual effects of each independent variable, because although the seed was taken from the same plants, each ANOVA used separate data sets from different experiments. The ANOVAs were conducted using the MGLH procedure of SYSTAT on an Apple Macintosh. The type III sums of squares were used to test for significance.

Results

Seed size

Mean seed mass varied two-fold and was positively correlated with the age of the seed parent (Fig. 1). The standard deviation of mean seed dry mass was inversely correlated with the age of the seed parent as was the coefficient of variation (Fig. 1). These relationships between mass and age suggested that while seed mass increased with plant age, variation in that mass among parents of the same age actually declined.

Seed and fruit Kjeldahl nitrogen concentrations were very low (generally less than 1%) in this parasitic plant. Because some of the data were pooled for nitrogen analyses and because of a limited number of samples, it was not possible to test if individual plants within an age category produced seed with more or less variation in nitrogen concentration than among age categories. Whereas seed mass correlated with the age of the seed parent, neither seed Kjeldahl nitrogen ($r=0.126$, $p>0.2$) nor fruit (pericarp) Kjeldahl nitrogen ($r=0.436$, $p>0.2$) concentrations were significantly correlated with parental plant age. When nitrogen is expressed on the basis of content (e.g., mg nitrogen per seed), however, there were positive and significant correlations with both the age of the seed parent and with the seed dry mass (Fig. 2). These results suggest that as the dry mass of seeds increased or as seed parents grew older, seed carbon to nitrogen ratio (C:N) remained constant.

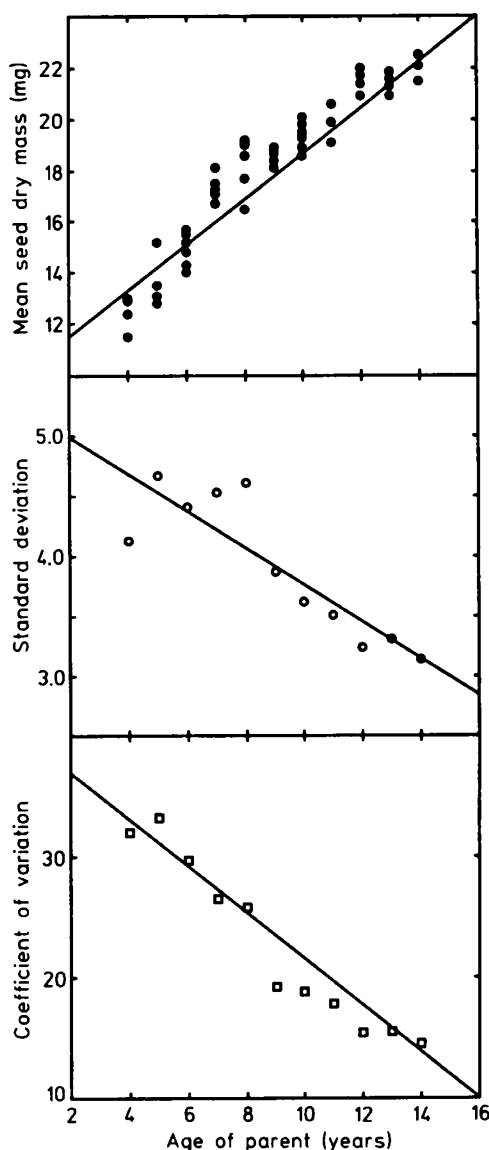


Fig. 1. Correlation between mean seed dry mass (top), the average standard deviation of seed dry mass (middle), and the coefficient of variation in seed dry mass (bottom) and the age of the mistletoe (*Phoradendron juniperinum*) seed parent. Fifty plants were sampled. The points in the lower panel are the means for all plants of that age. Regression equations: top; $y = 9.493 + 0.9663x$, $R^2 = 0.91$, $p = 0.017$, middle; $y = 5.296 - 0.1537x$, $R^2 = 0.77$, $p = 0.024$, bottom; $y = 41.041 - 2.052x$, $R^2 = 0.94$, $p = 0.011$

Germination and growth

Seed germination on the control medium varied between 20% and 86%; seed with greater mass and from older seed parents had the highest germination percentages in both years (Fig. 3). A summary of ANOVA and ANCOVA results demonstrate that single seed parents do produce seed of different germinability but that initial seed mass explains more of the variation in germinability than does age of the seed parent (Tables 1 and 2). These results suggest that smaller seeds may not have sufficient

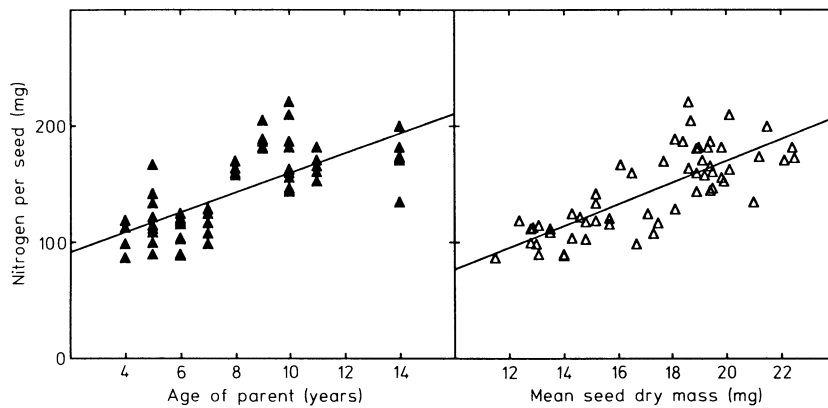


Fig. 2. The nitrogen content per seed as a function of the age of the seed parent (left) and the mean seed dry mass in *Phoradendron juniperinum*. Regressions for N per seed vs. age; $y = 74.25 + 8.49x$, $R^2 = 0.51$, $p = 0.037$, and for N per seed vs. seed dry mass; $y = 68.09 + 0.1773x$, $R^2 = 0.61$, $p = 0.029$

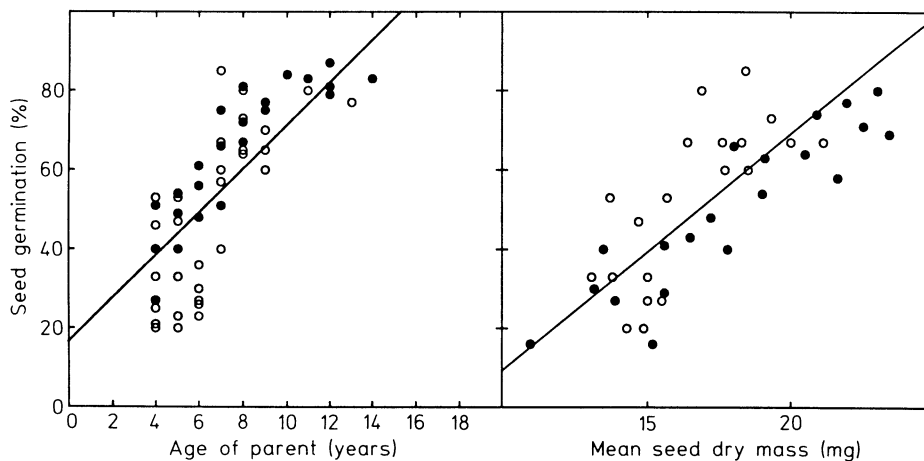


Fig. 3. Percent seed germination as a function of the age of the seed parent (left) and the mean seed dry mass in the xylem-tapping mistletoe *Phoradendron juniperinum*. The seed germination data were arcsine square root transformed before the regressions were performed. Regressions for % germination vs. age; $y = 17.71 + 0.062x$, $R^2 = 0.66$, $p = 0.024$, and for % germination vs. seed dry mass; $y = 8.89 + 0.058x$, $R^2 = 0.68$, $p = 0.019$

Table 1. Summary of one-way ANOVA partitioning variation in seed germination due to age of the seed parent, initial seed mass, individual source of seed

Variable	df	F	P
Age of Seed Parent	9	9.811	0.006
Initial Seed Mass	4	19.712	0.0001
Seed Source (individual)	48	0.093	0.217

resources to allow germination regardless of the age of the seed parent from which they come from.

Root radicle growth rates were highest in the heaviest seeds or those from older seed parents (Fig. 4). This was especially clear during the most active period of growth (between day 10 and day 40). During this period, roots that were produced from seed of greater initial mass (from older seed parents) had significantly steeper regression slopes (root radicle length vs. time) than did roots produced from seeds of lower mass (from younger plants; Table 3). In addition, the final root radicle length after 76 d of growth (the termination date of the experiment) was highly dependent on seed dry mass (Fig. 5). The ANOVA results from the root radicle growth experiments demonstrate that initial seed mass and age of the seed parent explain nearly equal amounts of the variation

Table 2. ANCOVA results of germination as a function of the age of the seed parent, the initial seed mass and the individual seed source. Table 2A are the results when age was the covariate. Table 2B are the results when mass was the covariate

Source	df	F	P
2A			
Covariate (age)	1	96.71	0.0002
Seed Mass	4	24.56	0.001
Seed Source	48	1.891	0.078
Mass \times Source		8.651	0.022
2B			
Covariate (seed mass)	1	113.45	0.0001
Age	9	5.67	0.042
Seed Source	48	2.091	0.064
Age \times Source		6.112	0.037

yet the individual seed source did not have a significant influence on root radicle growth rates (Table 4). In combination, these results demonstrated that root radicles produced from seed of greater mass not only grew faster, but also attained a greater total length. Such growth differences could confer an advantage to seeds of greater mass during establishment when autotrophic carbon gain is minimal or nonexistent and when access to nutritional resources of the host is not yet possible.

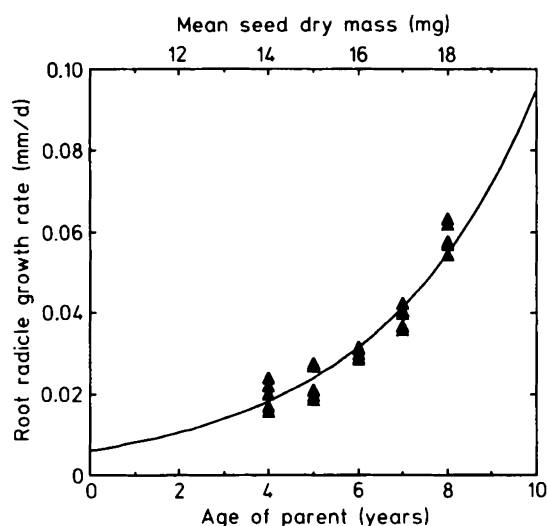


Fig. 4. Root radicle growth rate (mm per day) as a function of both the age of a seed parent and the mean seed dry mass from. Each point is the mean of 50 seeds taken from a single individual of that age with each particular age classes mean seed dry mass (+ or -0.5 mg). $y = 0.0059x 10^{(0.1203x)}$, $R^2 = 0.916$, $p = 0.016$

Table 3. Regression equation and the correlation coefficient for the relationship between root radicle length during the most active period of growth (day 10 to day 40) and time (in days) for seed taken from seed parents 4 to 13 years of age; sixty seeds from each age class. For all analyses, a Bonferroni multiple comparisons correction was performed

Age (yr)	Regression equation*	R ²
4	$y = -0.0396 + 0.0835x^a$	0.994
5	$y = -0.0636 + 0.1582x^b$	0.995
6	$y = -0.0853 + 0.2195x^c$	0.996
7	$y = 0.1656 + 0.3470x^d$	0.984
8	$y = 0.1367 + 0.4222x^e$	0.997
9	$y = -0.0511 + 0.5568x^f$	0.991
10	$y = 0.0777 + 0.6495x^g$	0.993
11	$y = 0.0786 + 0.6912x^{g,h}$	0.977
12	$y = 0.0333 + 0.7462x^h$	0.981
13	$y = 0.0501 + 0.8964x^i$	0.998

* Slopes that differed significantly from each other ($p < 0.05$) are noted with different superscripts

Germination and growth on foliage extracts

Juniper-leaf extracts affected both mistletoe seed germination and root elongation rates. Seed germination was significantly lower on the treatment medium than on controls (Table 5). Of the seeds that did germinate on the treatment media, significantly more formed the haustorial "disk". In separate experiments, when germinated seeds from 6- to 14-year-old parents were exposed to host-foliage extracts, the final root radicle lengths were significantly shorter than for control seedlings in all seedlings tested except for those measured from 6-year-old plants (Fig. 6; for clarity only the data from 6- and 11-year-old plants are shown). In addition, the juniper-leaf extract had the effect of reducing root radicle growth

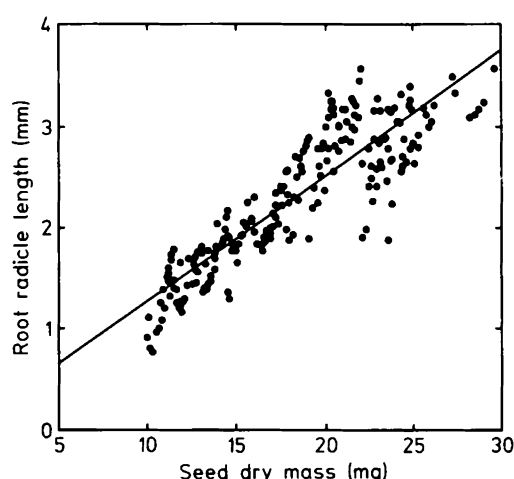


Fig. 5. Root radicle length (mm) as a function of the initial seed dry mass (mg) in *P. juniperinum*. $y = 0.0379 + 1.234x$, $R^2 = 0.784$, $p = 0.023$

Table 4. One-way ANOVA results partitioning variation in root radicle growth rate as a function of the age of the seed parent, initial seed mass, and the individual source of the seed

Variable	df	F	P
Age of the Seed Parent	9	21.76	0.001
Initial Seed Mass	4	23.45	0.001
Individual Seed Source	48	0.101	0.315

Table 5. The mean percentage of seed that germinated ($n = 56$ trials) and the average number of haustorial "disks" that formed (for 280 seeds, 10 seeds per trial) as a function of the type of growth medium. The control growth medium was agar + 1% sucrose and 1/4 strength Hoagland's solution. The treatment growth medium was the same as the control + 1% *Juniperus osteosperma* homogenized foliage. Values are means (s.d.); differences for both categories of data were significantly different (one-way ANOVA) at $p = 0.01$

	Control	Treatment
Percent Germination	57 (11)	23 (9)
Number of "disks"	17 (4)	54 (13)

Table 6A, B. ANCOVA results of germination as a function of age of the seed parent, initial seed mass, and the type of the germination medium (see text for details). **A** are the results when initial seed mass was the covariate. **B** are the results when age of the seed parent was the covariate

Source	df	F	P
A			
Covariate (seed mass)	1	168.77	0.0001
Age	9	13.811	0.004
Germination Medium	1	15.116	0.0008
Age × Medium	9	1.073	0.0461
B			
Covariate (age)	1	87.993	0.0003
Seed Mass	4	19.345	0.001
Germination Medium	1	14.237	0.002
Mass × Medium	4	0.975	0.0483

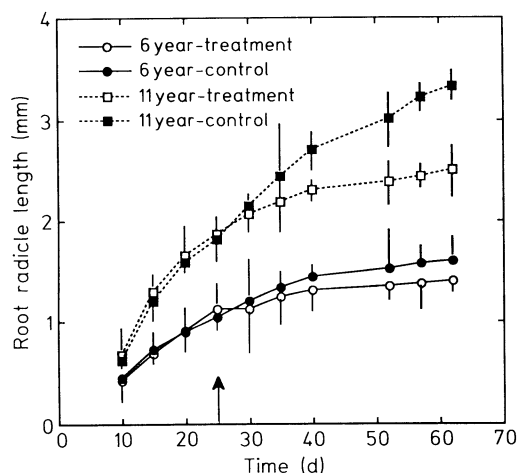


Fig. 6. Root radicle lengths (mean, s.d.) for seeds ($n=25$) obtained from 6 year old and 11 year old plants of *P. juniperinum* grown initially on a control medium and then transferred at day 25 to either a new control medium (closed symbols) or the treatment medium (open symbols) containing 1% host plant (*Juniperus osteosperma*) foliage. No statistical differences were seen between the control and treatment lines for 6 year old plants but significant differences were observed between the control and treatment lines compared from 11 year old plants

rates (doubling times; Fig. 7). ANCOVA results (with initial seed mass or age of the seed parent as the covariate) demonstrate that the age of the seed parent, the initial seed mass, the type of germination medium, and all two-way interactions significantly affected germination (Table 6). These results suggest that while seed germination may be significantly inhibited by host-foliage extracts, mistletoe seeds, and especially those from older parents or of greater mass, recognized the presence of the host and switched from a rapid, undifferentiated pattern of root elongation to a slower, more organized developmental pattern. This switch results in an anatomical structure capable of forming the union with the host plant's xylem (see below).

Scanning electron microscopy

To describe the developmental changes in root tissues from seeds germinated on control and juniper-leaf ex-

tract media, scanning electron micrographs were prepared from seedlings sampled at different stages of development. Marked differences were observed at different stages of development and in response to different growth media. Especially notable in the young seedlings on the control medium was the lack of a root cap and the presence of a loosely packed, undifferentiated mass of columnar-shaped cells at the distal end of the root radicle (Fig. 8A–C, E). Stomata were observed on the epicotyl near the collar of the seed just below the apical meristem (Fig. 8D). It is not known if these stomata were functional or if significant rates of gas exchange were taking place at this early stage of development. The undifferentiated cell mass in a 76-day-old root radicle growing on the control medium (Fig. 8E) was clearly different than the distal end of the root radicle growing on the treatment medium. Note the haustorial “disk” at the distal end of the root radicle when seedlings were grown on the treatment medium (Fig. 8F). This disk or holdfast is the anatomical union between the parasite and its host from which the endophytic system develops.

Discussion

Seed size – growth – establishment

Younger *Phoradendron juniperinum* plants produced seeds that were, on average, smaller than those from older plants. This result could arise if plants of all ages were selected to maximize seed number at the expense of seed size. However, because younger female plants would in general have a smaller total resource pool (C and N) from which to draw upon, they generally produce smaller seeds anyway, irrespective of how many are produced (Dawson et al. 1990b). Since seed mass is largely determined by the amount of carbon-based compounds present, lower overall photosynthetic carbon fixed per plant may, through its effects on the plants' carbon allocation program, be responsible for reduced seed mass in younger plants. In support of this idea, Schulze and Ehleringer (1984) observed that photosynthetic surface area of *P. juniperinum* was evergreen and that it increased exponentially as a function of age. Older stem tissues remained green, although it is not clear how active

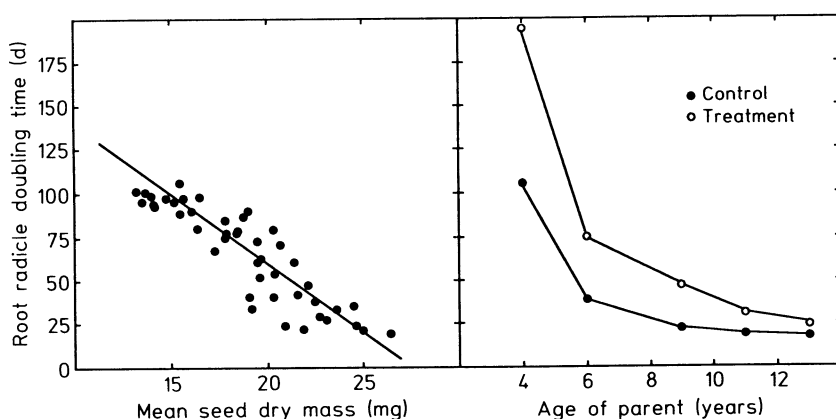


Fig. 7. Root radicle doubling time as a function of the initial seed dry mass (left) and the age of the seed parent on control (closed symbols) and treatment (open symbols) growth media. Regression for root radicle doubling time vs. seed dry mass; $y = 202.03 - 7.187x$, $R^2 = 0.799$, $p = 0.008$

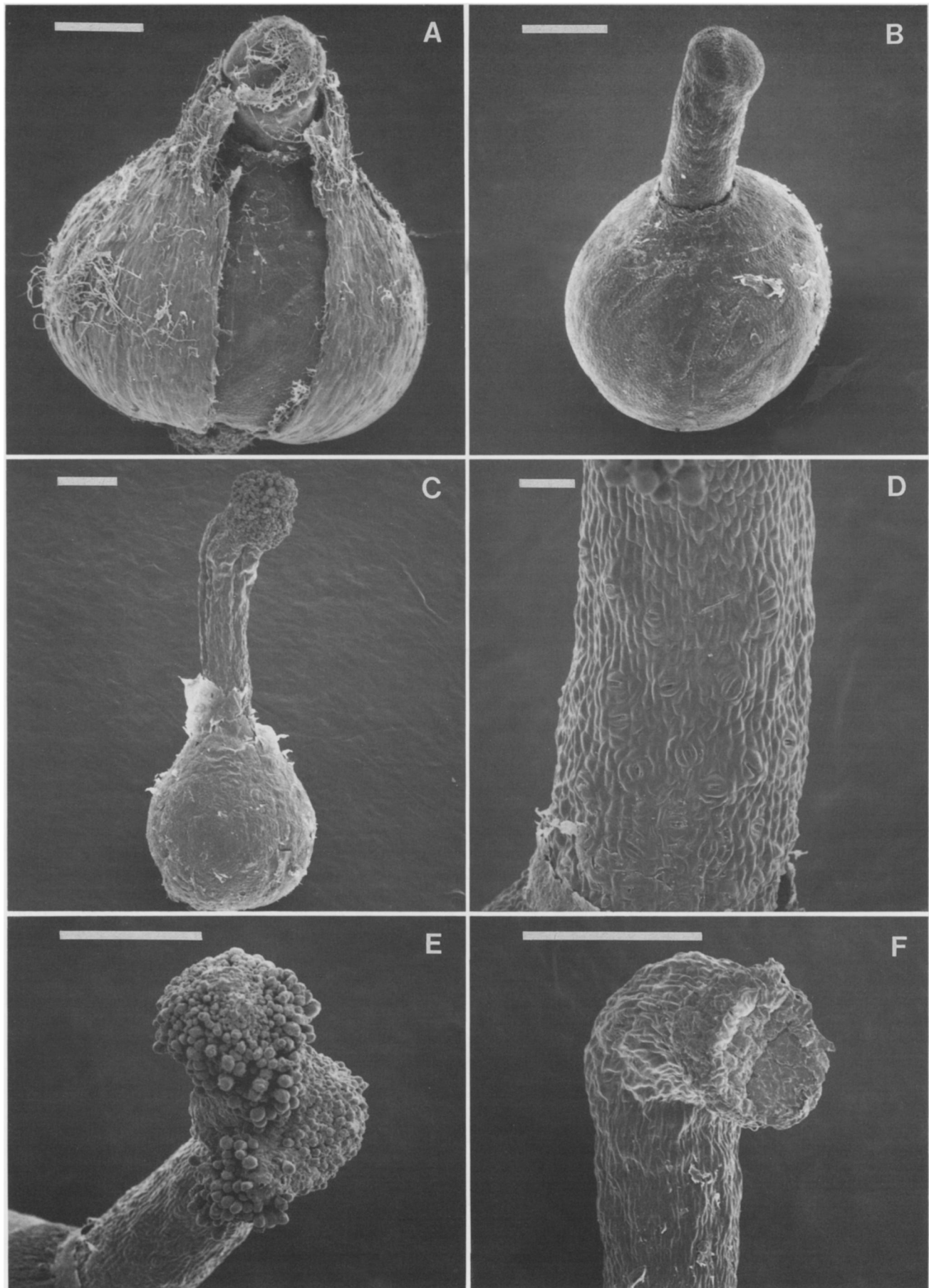


Fig. 8 A-F

older stems were in gas exchange activities. These factors would tend to increase the ratio of photosynthetic area per developing seed as a function of age. What is then not clear is that if larger seeds show an overall greater germination rate and growth performance as indicated by our results, why younger mistletoe plants have not shifted their allocation program towards producing fewer but larger seeds.

In a previous study (Dawson et al. 1990b) it was observed that both female and male mistletoe plants allocated an increasing larger proportion of their growth to reproduction as they aged. Thus, there is a positive association between the age, seed mass, and reproductive allocation, such that older plants not only make more seeds but larger and probably more successful seeds. This observation is somewhat different from that predicted in the clutch-size/constant-resource model of Smith and Fretwell (1974) or the two-resource model of McGinley and Charnov (1988) which both discuss a tradeoff between seed mass (size) and number if a plant's resource status remains constant. However, work by Begon and Parker (1986) and McGinley (1989) predict that a positive correlation between total allocation to reproduction and offspring size should exist if seedling fitness depends upon clutch size. The data shown here support this prediction, especially if seed dispersal is dependent upon plant size which is very likely in this mistletoe species (see below). Further, recent observations on salamanders (Semlitsch 1985), the common toad (Reading 1986), and mosquito fish (Meffe 1987) exhibit similar patterns to the results we show here for a hemiparasitic plant: larger or older females produce larger as well as more offspring.

It has been shown that morphological constraints imposed by pleiotropy in a plant's developmental system could have an influence on other correlated traits (see McGinley et al. 1987). For example, the size of plant parts arising from the same meristem may be positively correlated such that older, larger plants, produce larger seeds (Sinnott 1921). The problem of allometric influences on the size of seeds is beginning to receive more attention and in some species has been shown to be very important (Thompson and Rabinowitz 1989). In fact, we know from our previous work on *P. juniperinum* (Dawson et al. 1990a) that older plants have a greater number of branch orders. In other species, seed mass has been shown to correlate with branch order, with mass decreasing as the number of branch orders increases (M. McGinley, personal communication). However, we see just the

opposite trend, with mean mass increasing with age of the parent and the number of higher branch orders, a pattern that may result from the fact that mistletoes do not have to allocate as much carbon to "roots" and thus may be able to divert it to reproductive activities. What we do not know, however, is how seed mass varies with branch order within a plant. In addition, seed mass or size could be influenced by the efficiency, rate, or duration of phloem unloading imposed by constraints of a plant's modular growth form and hence its vascular architecture (Watson and Casper 1984). As suggested by McGinley et al. (1987), different seed sizes may arise because seed provisioning among different modules are exposed to an array of different microenvironments that in turn affect the rate and duration of seed filling. We feel that closer examination of the carbon and nitrogen allocation characteristics in relation to plant age (morphology) and unique to the hemiparasitic life history may provide some insights as to why some of the unusual relationships we observed exist in *P. juniperinum*. The way in which the data from this study were collected do not allow us to properly address these issues.

Chemical stimuli – growth – germination

In mistletoes, we would expect that natural selection has favored rapid seed germination and high seedling growth rates to avoid a lengthy exposure to desiccating environmental conditions when the parasite has no access to additional water. The results of our growth experiments on media containing juniper-leaf extracts might initially be viewed as counter to this idea, since root radicle growth rates were reduced under treatment conditions. This observation was especially pronounced in heavier seeds. However, the observations of increased haustorial disk formation under treatment media suggests that a form of host-plant recognition had taken place in these, as in other parasitic plants (Stewart and Press 1990). An accelerated growth rate was no longer necessary when the point of union with host is near. Greater haustorial disk formation in relation to a variety of host-plant stimuli has been observed in other mistletoe investigations (Baldwin and Speese 1957; Atsatt and Strong 1970; May 1971; Thomson and Mahall 1983; Clay et al. 1985; Kuijt 1986). Further, research on several root parasites suggests that chemical extracts can be obtained from host plant root tissues which may elicit a host-parasite recognition process (Cook et al. 1966; Tinnin et al. 1971; Johnson et al. 1976; Saghir 1979; Atsatt 1979, 1983; Stewart and Press 1990). In *P. juniperinum*, although seed germination and root growth were negatively affected by juniper-leaf extracts, a clear and significant developmental shift towards the establishment of the host-parasite union was observed. A host-parasite recognition response of this type could have been selectively advantageous in *P. juniperinum*, and in turn could have influenced patterns of development. It is also possible that reduced germination reflects a sort of chemical defense of the juniper and that haustorial disk formation indicates success in those seedlings to overcome the de-

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Fig. 8A–F. Scanning electron micrographs of the stages of seed germination and development in *P. juniperinum*. Notable features are, a large seed and emerging root radicle that lacks a root cap (**A, B**), an undifferentiated mass of columnar-shaped cells at the terminal end of the root radicle when seeds were germinated on the control medium (**C, E**), the presence of stomata on the epicotyl just above the seed collar-root radicle interface (**D**), and the presence of the haustorial "disk" (the parasitic union) at the terminal end of the root radicle when seeds were germinated or exposed to a growth medium containing a 1% juniper-leaf extract (**F**). Scale bar = 500 μ m for all pannels except pannel **D** where it is equal to 100 μ m

fense. This could be tested by germinating seeds on extracts from an array of individuals or individuals from different populations to see if the host junipers vary in their susceptibility to mistletoe infection in a way predicted by the *in vitro* tests conducted here.

Carbon – nitrogen ratios

Several authors have demonstrated that the growth rate of roots, shoots, or entire seedlings was tightly correlated with seed or fruit mass (Harper and Obeid 1967; Weis 1982; Zimmerman and Weis 1983; Stanton 1984; Wulff 1986; Marshall 1986; but see Choe et al. 1988). Increased vigor may result from larger or heavier seeds containing a greater total resource pool that may directly influence growth performance (Wulff 1986) or allow a seedling the opportunity to draw on those resources for a longer period of time. Our data indicated that mistletoe seeds of greater mass have more total resources, but not significantly higher concentrations of two key components, carbon and nitrogen. Thus, the C/N ratios remained constant with seed size.

McGinley and Charnov (1988) have extended the Smith-Fretwell tradeoff model to a two-resource case (carbon and nitrogen). Under the simple assumptions of their model (1) optimal seed size (carbon) should be positively correlated with the C/N ratio available for investment in offspring, (2) there should be a negative correlation between seed size and seed nitrogen content, and (3) seed nitrogen content should be negatively correlated with the C/N ratio. These predictions are based on previous observations that carbon is typically invested into seeds after anthesis (Pate and Layzell 1981; Bewley and Black 1985), whereas nitrogen is typically allocated to seeds prior to anthesis (Pate and Layzell 1981). Our data cannot directly address the first prediction of the McGinley-Charnov model. However, the second and third predictions of the model appear not to be supported by the mistletoe seed data presented here. This could result for at least two reasons. First, the model is static in nature, assuming that the amount of a particular resource is fixed before it is allocated. Second, resource allocation is a dynamic process and seed and fruit development are relatively slow in mistletoes (10 to 14 months to maturity). Further, final seed mass has been shown to be significantly influenced by the rate and duration of seed filling for many plant species (Kaplan and Koller 1974; Jones et al. 1984). For these reasons or perhaps others one might not expect the models predictions to be upheld.

Seed size – seed number tradeoffs and the hemiparasitic life history

Variation in seed mass, size, and number significantly affect a wide range of physiological, ecological, and demographic processes in plants (reviewed in McGinley et al. 1987). It has been proposed for a broad array of species that seed size or mass appears to be so tightly linked to life history traits, such as age or lifespan, that

increases in seed size or mass are almost always associated with decrease in seed number (Smith and Fretwell 1974; Silvertown 1981). We found no evidence for such a tradeoff in *P. juniperinum*. The observation that older mistletoes produce both larger and more seeds may be a product of the hemiparasitic life history in that more resources can be allocated to reproduction since only limited resources are allocated to root-like structures. Though we have no direct evidence that such a trade-off exists, we have observed that the fraction of carbon resources allocated to reproduction continually increased with mistletoe age in both male and female plants (Dawson et al. 1990b).

Since older plants produce larger and more seeds which have a performance advantage relative to younger plants, we might expect a disproportionate amount of establishment from seed derived from older individuals within the population. This in turn will have important consequences on the genetic structure of mistletoe populations as well as age-specific effects on the demography of *P. juniperinum*. Though we cannot rule out the possibility that the positive correlations between seed mass, age, germination, and seedling growth may be influenced by adult size, we feel that selection acting at the colonization and establishment phase are most important and what have ultimately influenced these correlated characters.

Given the size advantage of seeds from older mothers, two reasonable questions to ask are “if older plants produce seeds with greater fitness, why don’t young plants produce fewer, large seeds” and “if older plants produce seeds with greater fitness, why not delay reproductive activity until large seeds can be produced?” Although it appears that photosynthetic area may ultimately limit seed size in younger plants, it is not at all clear why fewer seeds are not produced or why variation decreases as the seed parent ages. This kind of pattern could result from developmental patterns. That is, if carbon gaining capacity increases faster than the number of reproductive modules as plants age, then young plants may be constrained to produce only small seeds. Of the observations presented here, it is this area that remains the most illusive and certainly begs further empirical and theoretical attention. On the second question, some insight can be gained by considering the factors responsible for adult mistletoe mortality. Death of mistletoes is thought to be associated with either of two infrequent and unpredictable climatic events: extreme low winter temperature events or extensive drought periods (see Dawson et al. 1990b for a full discussion). Mortality during these climatic events appears to be high, affecting individuals in all age classes. Whereas a selective advantage is associated with offspring produced by mistletoes of older age classes, the risk of producing no offspring by delaying reproduction and possibly experiencing cold temperatures and/or drought is also high. In other words, in this mistletoe species, the climatic extremes may be frequent enough at this site to select against delayed reproduction, even though fitness of the offspring produced from younger plants may be relatively low.

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