Folia Geobot
DOI 10.1007/s12224-013-9187-8

Interaction Between “Safe *Sites*” and “Safe *Sides*” for Germination of *Neurada procumbens* (Neuradaceae) in the Middle East

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Abstract The seed bank of *Neurada procumbens*, a prostrate annual common in sandy desert habitats, is vulnerable to surface disturbances. Moreover, its seeds are highly heteromorphic, having both spinose and non-spinose sides, which affects both the vertical distribution of seeds and diaspores, and the precise position in which they lie in the soil. Here we explore the ecological implications of two factors, “safe *sites*” within habitats and seed orientation or “safe *sides*”, on seedling establishment and the seed bank. In three natural populations, the vertical distribution of buried, germinable diaspores decreased with soil depth. Most diaspores occurred at the surface (0–1 cm below the surface), while none were observed below 10 cm. Seedling emergence decreased with depth and reached zero in diaspores below 5 cm. Seedling emergence also proved highly sensitive to the orientation of diaspores in soil. Diaspores lying on their smooth side with the spinose upper side directed upwards reached the highest seedling emergence of 61 %. Root length ranged from 10 to 16 cm, and the root to shoot ratio varied between 22.8 and 30.3 cm, depending on diaspore size. Diaspores near the surface may constitute a more transient component of the seed bank, as few of these remained viable for more than a year. Persistence of *Neurada* seed banks seems to depend not only on vertical movement of diaspores among habitat “safe *sites*” but also on different seed position alternatives.

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Keywords Asymmetrically spinose fruits · Depth of burial · Diaspores · Seed bank persistence · Seed germination · Seed output · Seed/diaspore orientation · Seedlings

Introduction

Despite their short lifespan, desert annuals possess many species-specific adaptations. Seeds are typically affected by a diversity of factors including dispersal between sites with suitable habitats as well as horizontal and vertical movement in the soil or substrate. Other factors include temperature, light and moisture fluctuations as well as soil characteristics and surface perturbation (Baskin and Baskin 1998).

The spatial distribution of seed-containing fruits (or diaspores, *sensu* Gutterman 1994) in soil is heterogeneous and constitutes part of a fine-scale interaction between seed architecture and soil. Spatial heterogeneity of soil seed banks in arid and semi-arid environments is mainly driven by heterogeneity in soil characteristics and vegetation cover. This results in distinct patterns of seed accumulation and distribution in soil (Reichman 1984; Thompson 1987; DeFalco et al. 2009; Quevedo-Robledo et al. 2010; Dreber and Esler 2011). Habitat disturbances and soil perturbation favor the loss of some micro-sites while creating others and facilitates exploitation of propitious conditions whenever they may arise (Grime 1979; Hobbs and Atkins 1988; Kinloch and Friedel 2005a,b; Liu et al. 2011).

The seed bank of *Neurada procumbens* is particularly vulnerable to surface disturbances (Gutterman 1994). Perturbation by both wind and animal action mixes the topmost and sub-surface layers, sometimes changing the qualities of the soil micro-relief (Harper et al. 1965; Eckert et al. 1986; Benvenuti 2003; Poorter 2007; Hernandez and Sandquist 2011). Effects of burial depth have been examined in many species (e.g., Stamp 1984; Benvenuti et al. 2001; Boyd and Van Acker 2003; Chantre et al. 2009; Saatkamp et al. 2011), but fine-scale effects of the angle and orientation at which seeds or diaspores are positioned in the soil have not been much studied.

We focus on two aspects of seed germination and establishment in *N. procumbens*: "safe *sites*" (*sensu* Harper 1977) and "safe *sides*". "Safe *sites*" represent local micro-sites within sandy habitats which provide conditions especially suitable for germination of seeds and establishment of seedlings (see Harper 1977; Schupp 1995). Most species favor particular micro-environmental conditions, for example, optimal burial depth of seeds in soil, constituting individual "safe *sites*" (e.g., Harper 1977; Rühl and Schnittler 2011). For *N. procumbens*, favorable micro-sites reduce drought, high temperature and insolation stress, and facilitate seed germination and seedling establishment.

In species having highly heteromorphic seeds or diaspores, for example, *N. procumbens*, it seems likely the particular orientation of the seed may be significant. We look at such a possible "safe *side*" effect and suggest that successful seedling establishment and seed bank persistence may be controlled not only by vertical movement of diaspores to "safe *sites*" but also by seed orientation relative to the "safe *side*" position. We probed the effect of orientation on germination experimentally by placing diaspores in an array of positions.

Once diaspores become separated from the plant, they are controlled by the physical structure of the soil surface and external forces. Deposition of diaspores containing viable seeds in a local "safe *site*" does not guarantee successful seed germination and seedling establishment. We hypothesize that diaspores need to be lying on their "safe *side*" and discuss possible effects of orientation on seed bank persistence.

Material and Methods

Study Sites

Plants were studied in three natural populations. These were located in the Thumamah National Park about 70 km west of Riyadh city (Saudi Arabia), Ras Sidr in South Sinai (Egypt) and Wadi Elnatroun by the Cairo-Alexandria desert road about 75 km west of Cairo (Egypt). The climate at all the sites is arid, and their sandy soils are characterized by a low content of organic matter and high concentrations of carbonates (Table 1).

All three sites are subject to occasional anthropogenic disturbance. At Thumamah, perturbation results from recreational land use activities. The Ras Sidr site is exposed to mild grazing and trampling activities. At Wadi Elnatroun, the site is located at the edge of newly reclaimed farmland and is disturbed by various agricultural practices. Natural disturbance at all three sites include regular, strong wind and rapid water run-off. All these factors may cause active accretion and deflation of moving sand and are likely to influence the depth of burial and movement of diaspores in soil.

Table 1 Study sites characteristics in Thumamah, Ras Sidr and Wadi Elnatroun. The source of climate data: CLAC (2010) and http://www.timeanddate.com/weather/saudi-arabia/riyadh. Soil analysis data expressed as mean ± SD. Values of soil variables in the same row marked by different letters are significantly different ($P < 0.05$)

Character	Thumamah	Site Ras Sidr	Wadi Elnatroun
GPS location	N 25°16′17.4″ E 46°38′58.8″	N 29°31′51.8″ E 32°49′52.1″	N 30°21′15.2″ E 30°26′55.1″
Elevation (meter a.s.l.)	671	66	115
Annual rainfall (mm)	101.7	63.5	58.2
Mean max annual temperature (°C)	34.2	28.7	28.8
Mean min annual temperature (°C)	18.9	14.9	14.7
Mean annual evaporation (mm/day)	15.6	8.4	9.5
Soil characters:			
Sand (%)	65.3 ± 4.26^{a}	58.6 ± 5.08^{a}	51.4 ± 2.36^{b}
Silt (%)	14.8 ± 2.20^{a}	11.6 ± 0.79^{a}	17.5 ± 2.85^{b}
Clay (%)	6.2 ± 1.52^{a}	3.5 ± 0.8^{b}	10.2 ± 2.06^{c}
pH	7.7 ± 0.22^{a}	7.4 ± 0.20^{a}	7.1 ± 0.15^{a}
Electrical conductivity (μmhos/cm)	739 ± 38^{a}	1185 ± 52^{b}	1658 ± 61^{c}
Organic matter (%)	1.2 ± 0.15^{a}	0.5 ± 0.12^{b}	1.8 ± 0.15^{a}
$CaCO_3$ (%)	26.9 ± 2.47^{a}	22.2 ± 2.73^{b}	16.5 ± 1.60^{c}

Study Species

Neurada procumbens L. (Neuradaceae) is a ruderal, annual herb. Its distribution range spans from North Africa and the Mediterranean region across the Middle East to Afghanistan, Pakistan and north-western India (Marwat and Siddiqui 2013). Typical habitats include inland dunes and sand sheets, alluvial and interdunal plains, wadis and rough mountain terrain. The species has also been reported as a naturalized weed in Australian deserts (Albrecht et al. 2002).

Populations occur in open, sandy habitats that experience frequent disturbance events, seasonal droughts and high temperatures. Typically, dry fruits (known as diaspores) are blown by wind or lie on the surface of the sand. If trodden on, their spines attach to the feet of animals (vehicle tires) and may be carried over considerable distances before falling loose (Farghali 1940). Individuals exhibit interesting rapid germination followed by rapid development and flowering.

When rain falls, one or several of the ten or so seeds in a diaspore germinate, rapidly sending a fine tap-root down into the moist sand. If there is a dry period during this event, the tap-root withers, and the seedling dies. After subsequent showers, however, further seeds in the same diaspore germinate, and one or more may grow successfully. This allows the species to germinate after the first showers and benefit from a long growing period (Vesey-Fitzgerald 1957; Danin 1983; Gutterman 1994). The active phase of the plants' life span is brief and limited to a single growing season and one reproductive session. The time to reproductive maturity is variable, ranging from weeks to months, and depends on water availability (Hegazy and Ismail 1992; Hegazy and Kabiel 2010; Hegazy et al. 2013).

The species is characterized by persistent hard diaspores, each containing 8–10 seeds. These remain encapsulated within the diaspore. Seeds germinate while still inside the hard, discoid pericarp. Both radicles and plumules perforate the pericarp and grow downwards and upwards, respectively. Roots of all seedlings pass through the central hole marking the point of attachment of the diaspore stalk. After germination, the old, spiny, flattened hypanthium often persists as a kind of girdle around the plant base at the junction of the stem and root (Fig. **1**; Turki 2007). Mature diaspores are dry and leathery, with a rough, spiny upper and smooth lower surface. The spinose upper surface facilitates diaspore dispersal by clinging to animal fur and human shoes and clothing. The entire structure is light and can be blown by the wind. Mature diaspores range from 0.5 to 2.25 cm in diameter.

Soil Analysis

For determination of field soil physical and chemical characters, five samples were collected from each locality (samples ca. 200 g) and air dried at the laboratory. Determination of soil characteristics followed Hegazy et al. (2009).

Seed Output

At the senescence / diaspore dispersal stage (end of April 2010), mature dry diaspores were collected from the three sites. The seed output measurements were performed in the same plots as the seed bank study. Estimates of the number of total diaspores per square meter (five replications) were calculated based on all individuals in the plot, and these were stored for further investigation.

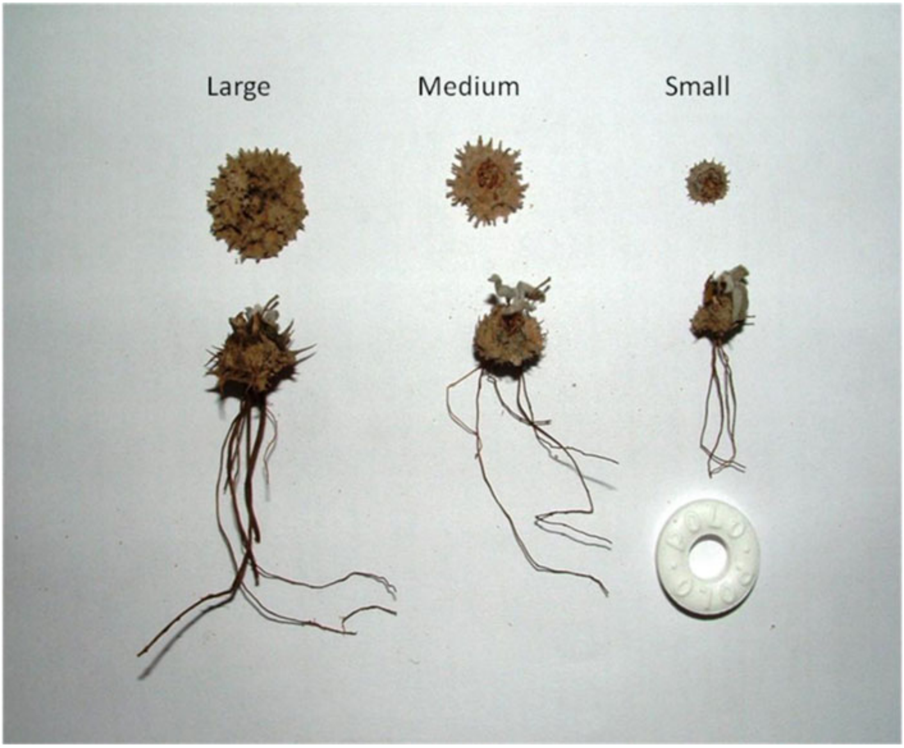

Fig. 1 Seeds of *Neurada procumbens* and seedlings emerged from large-, medium- and small fruits (diaspores). Roots of all the germinating seedlings in a diaspore pass through a central hole marking the point of attachment of the diaspore stalk. After germination the flattened ring-like diaspore skeleton persists around the plant at the junction of stem and root. In this photo, more than one seedling has emerged from each diaspore. Photographed with a 2 cm tablet for scale

In the laboratory, diaspores were sorted into three size classes: (1) large, exceeding 20 mm in diameter; (2) medium-sized, 10–20 mm in diameter; and (3) small, less than 10 mm in diameter (Table 2). The fruit weighed between 84 and 239 mg. The diaspores were examined for insect damage, and in any way damaged material was excluded. The seed output was then estimated as the number of intact diaspores per square meter.

The extent of insect damage was estimated for each size class separately. Samples of 50 diaspores were opened using a needle, and seeds were observed under a magnifying glass or binocular microscope. Seed insect damage occurred mostly during the seed ripening stage, but many seeds escaped damage and remained intact.

Table 2 Fruit (diaspore) weight and diameter in different size classes (mean ± SD). Values in the same column marked by different letters are significantly different ($P < 0.05$)

Fruit size	Fruit weight (mg)	Fruit diameter (mm)
Large (more than 20 mm)	239.0 ± 27.76^a	21.4 ± 1.34^a
Medium (10–20 mm)	145.8 ± 30.40^b	15.8 ± 3.27^b
Small (less than 10 mm)	84.0 ± 8.37^c	7.0 ± 1.58^c

Seed Bank

Since seeds of *N. procumbens* are not released from diaspores, the term "seed bank" in this paper is used to denote diaspores at and beneath the soil surface, in particular those which are viable and capable of germination (see Graham and Hutchings 1988). Five randomly located plots (1 × 1 m) were selected at each study site for sampling of seed banks at the end of the summer dry season in September 2010. The plots were randomly selected and taken from the same habitat type at each sample site. The distance between plots ranged from 25 to 50 meters (the same plots were used for estimates of seed output). Every plot was further divided into 25 subplots (20 × 20 × 10 cm depth). Five subplots from each plot were sampled randomly. Profiles were carefully scraped into separate layers: (1) 0 cm (surface layer); (2) 0–1 cm; (3) 1–2 cm; (4) 2–5 cm; and (5) 5–10 cm depth. The depth of ten centimeters was considered sufficient, as preliminary studies had indicated no *Neurada* diaspores at greater depths. Five random soil samples were scraped from a depth of 10 to 20 cm and found not to contain any viable *N. procumbens* diaspore skeletons, only remains of decayed diaspores. Soil from each layer was pooled per plot; the overall number of subsamples was 25 per layer, representing five plots per site. In the field, soil was sifted through 2 mm mesh screens, and *N. procumbens* diaspores were collected and stored for further investigation.

Diaspores were inspected and sorted manually with the aid of a magnifying lens and binocular microscope. Empty fruits and skeletons were excluded. All diaspores containing seeds were counted as present in the seed bank. To estimate the germinable seed bank, the number of diaspores was counted in 20 × 20 × 10 cm subplots and scaled up to the number of diaspores per square meter. To test seed viability, five random samples of 50 diaspores, each constituting a total of 250 diaspores, were wetted overnight on a paper towel. The seeds were then tested by the tetrazolium viability test (Malone 1967).

Seedling Emergence and Establishment

Mature diaspores collected from Thumamah in April 2010 were used in subsequent seed germination / seedling emergence tests. Tests were performed on large, medium and small diaspores. A total of 250 intact diaspores from every size class was used. These were separated into five replications, each comprising 50 diaspores. Tests were performed in small plastic pots filled with sandy soil collected from the Thumamah study site (see Table **1** for soil characteristics). Soil was sampled from depths of 0–20 cm. To avoid desiccation around the diaspores, the pots were watered regularly with tap water every other day using a hand-held sprayer. Diaspores were treated as seeds, and seed germination counts were carried out once per diaspore (i.e., counted as one seed even when multiple germinations occurred in a diaspore). Germination was evaluated as seedling emergence as the appearance of cotyledons above the soil surface.

We assumed no seeds get released from diaspores early, as the ring-like diaspore skeleton was found to persist tightly around the plant. Once one or more seeds germinated in a diaspore, further seeds mostly did not germinate, and the diaspore decayed. When assessing germination rates, we treated the infrequent multiple germinations from one fruit as single germination events; however, the total number of seedlings is their true count (Fig. **1**).

Germination tests began on 20 January and ended on 30 March 2011, when no further seedlings had emerged for ten consecutive days. The experiments were carried out at the King Saud University Botanic Garden. Air temperature ranged from 17.2 to 33.5°C.

Seedling establishment (i.e., emergence and growth) was determined using samples produced in the emergence experiment. Successful seedling establishment was defined as the appearance of the first two post-cotyledon leaves (Fig. 1). Data were obtained for seedlings grown from diaspores of the large, medium-sized and small size class (collected at the Thumamah locality) and sown at the depth of 0.5 cm. Seedling age at time of harvest was 30 days. Five seedlings produced from every diaspore size class were randomly selected and carefully excavated, and the length of each root and shoot was measured. The seedlings were then oven-dried at 85°C to determine their total dry mass.

Depth of Burial

Plastic pots (5 × 5 × 10 cm depth) were used for germination tests. The pots were filled with oven-sterilized soil from the Thumamah study site. Diaspores representing the medium size class collected from the Thumamah site (selected at random from the diaspore stock) were sown as one diaspore per pot into the substrate at depths 0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 cm. Diaspores were sown lying horizontally on the smooth lower side with the spinose side facing upwards. Five replications of 50 diaspores each were used for every sowing depth.

The pots were watered with tap water every other day or as needed to keep the soil moist. After about two weeks (the tenth irrigation), 50 ml of 0.01 M solution of potassium nitrate and 50 ml of 6.5×10^{-4} solution of gibberellic acid were added, followed by tap watering for possible activation of dormant seeds (see DeFalco et al. 2009). Germination was monitored daily and every other day toward the end of the experiment. After termination of the experiment, diaspores were excavated and examined. Emergence of the radicle was taken as a sign of germination. Ungerminated diaspores were tested for viability. Any viable but ungerminated seeds were considered dormant. The viability of ungerminated seeds was 62.8±7.23 %.

“Safe Side” Test

This test was carried out in plastic pots (5 × 5 × 10 cm depth) in the same soil and under the same germination conditions as used to test the depth of diaspore burial. Diaspores representing the medium size class collected from the Thumamah site were selected at random from the diaspore stock and then sown one diaspore per pot. Diaspores were sown at the optimal depth for seedling emergence (0.5 cm).

Diaspores were sown lying in different positions in the soil (Fig. 2): (1) abaxial position, where diaspores were lying horizontally on the flat, smooth lower side; (2) inverted position with diaspores lying horizontally on the spinose upper side; (3) vertical position with diaspores positioned vertically on the side; (4) inclined position with diaspores positioned lying inclined at ca. 45° with flat, smooth side down; and (5) inclined position with diaspores positioned lying inclined ca. 45° with spinose side

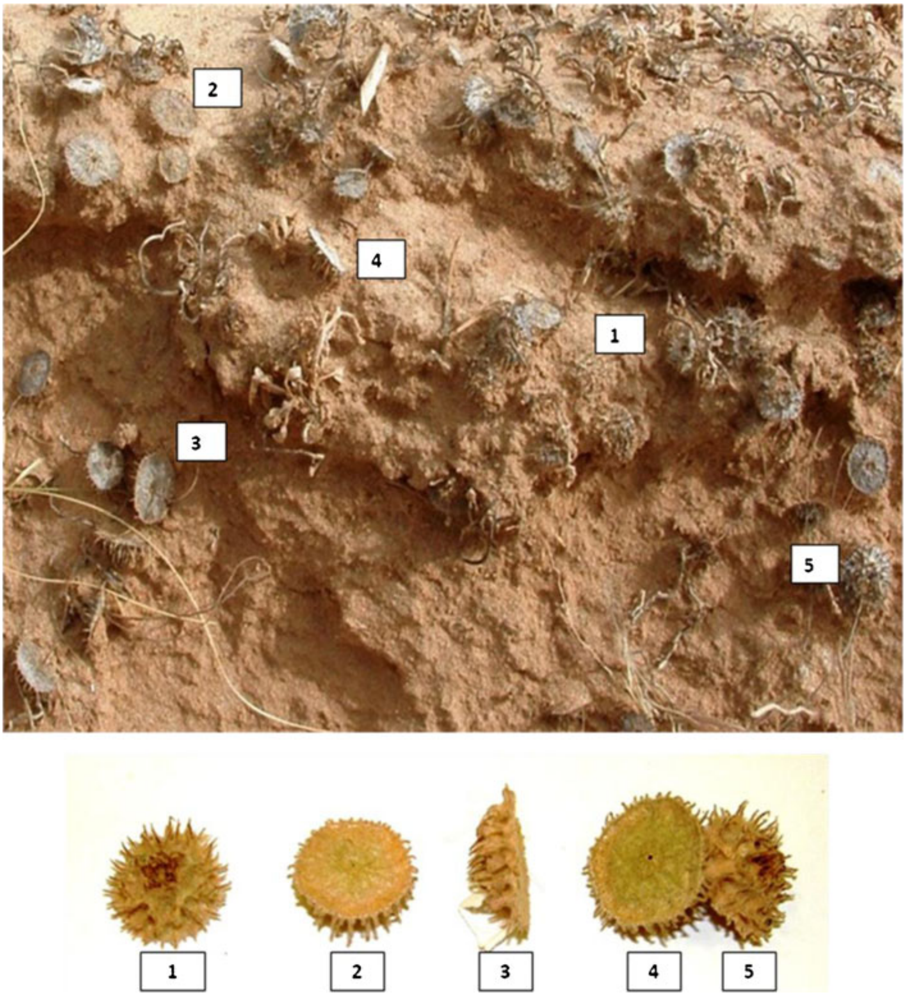

Fig. 2 Orientation of *Neurada procumbens* diaspores in the seed bank at the Thumamah study site. (1) Abaxial position (with diaspores lying horizontally on their smooth lower side); (2) inverted position (upside-down) with diaspores lying horizontally on the spinose upper side; (3) vertical position, diaspores vertically on their side; (4) inclined position diaspores positioned lying inclined at different angles, smooth side upwards; and (5) inclined position with diaspores lying inclined spinose side upwards

down. Each test concerning diaspore orientation was carried out on 50 diaspores in five replications (i.e., a total of 250 diaspores).

Statistical Analysis

We tested the effects due to the site, diaspore size and depth of burial, and their interactions on seedling growth criteria as response variables by analysis of variance using SPSS 15 for Windows (SPSS Inc., Chicago, USA). By a split-plot ANOVA, performed in MSTAT-C v. 2.10, we analyzed seed bank and seed rain data as response variables; percentages of seedling emergence and the root-shoot ratio were arcsine square-root transformed.

Results

Seed Output

Mean viable seed (diaspore) output varied significantly among sites and among diaspore size classes (Fig. 3, Appendix 1). The highest viable diaspore output was observed at the TH site: 512±18.5 diaspores per square meter, compared to values of 340±10.6 and 395±15.7 diaspores per square meter (mean ± SD) at the RS and WE sites, respectively. Medium-sized diaspores were the most common at all three study sites (collectively exceeding 50 % of the total number of diaspores, over all sizes). Larger diaspores had the lowest levels of seed output among diaspore size classes. On the other hand, diaspores of this size class suffered the most insect damage.

Insect-damaged diaspores amounted to 11.4±2.4 %, 25.3±2.8 % and 27.8±3.7 % (mean ± SD) of the total seed output at RS, WE and TH sites, respectively. Insect-damaged diaspores averaged 68.2±4.5 % of the total seed rain at the RS site, for medium-sized diaspores, and reached 49.7±3.2 % and 39.2±3.4 % (mean ± SD) at the TH and WE sites, respectively. At all three sites, the smallest diaspores were the least damaged by insects.

Seed Bank

At the three study sites, significant differences were found among the three diaspore size classes depending on soil depth in the top 10 cm (Fig. 4, Appendix 2). The mean total germinable seed bank in the top 10 cm amounted to 346±41.1, 261±11.8 and 324±26.7 diaspores per square meter (mean ± SD) at the Thumamah (TH), Ras Sidr (RS) and Wadi Elnatroun (WE) sites, respectively. Medium-sized diaspores dominated the seed bank at the TH and RS sites, while small diaspores were most common at the WE site.

The distribution of diaspore size classes with depth showed that medium-sized diaspores reached the highest mean values in the surface soil layer (reaching 68.2±6.1, 17.8±2.7 and 38.0±3.9 percent (mean ± SD) at the TH, RS and WE sites, respectively). At all depths, from just below the surface down to the depth of 10 cm, both medium-sized and small diaspores attained higher values than did large diaspores, except at the WE site where no viable seeds were found below 5 cm depth.

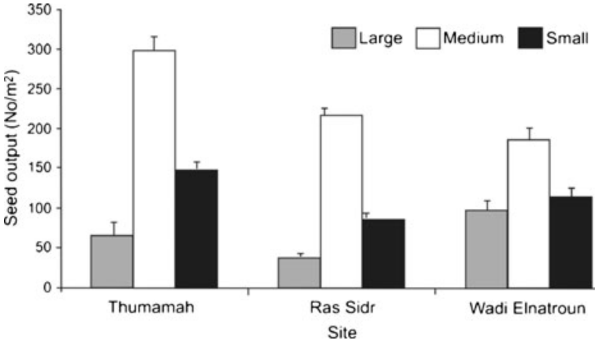

Fig. 3 Seed output of *Neurada procumbens* expressed as the number of diaspores of three diaspore size classes produced per square meter at three study sites (mean ± SD)

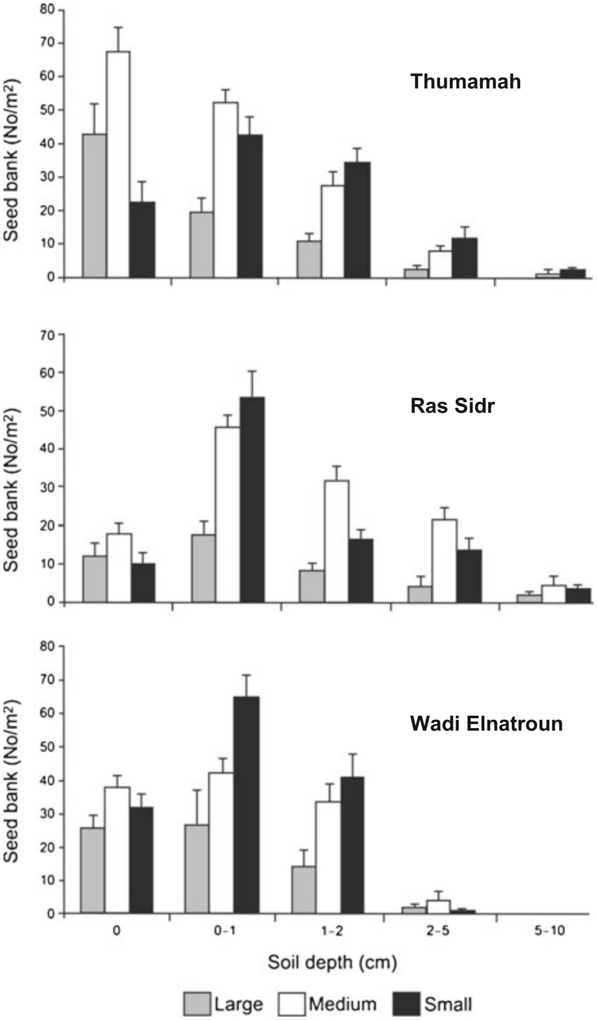

Fig. 4 Seed bank of *Neurada procumbens* at three study sites, Thumamah, Ras Sidr and Wadi Elnatroun expressed as the number of diaspores of three diaspore size classes found at different soil profile depths (mean ± SD)

The vertical distribution of buried seeds steadily decreased with soil depth. Comparing different diaspore size classes, significantly higher proportions of large diaspores were found in the upper soil layers. Alternatively, small diaspores were found at higher frequencies in the deeper layers of the profile. At the TH site, the percentage of seeds decreased from 38.8 % at the surface to 0.9 % at 5–10 cm. For the RS site, the percentage decreased from 44.7 % at depth 0–1 cm to 3.6 % at 5–10 cm. At the WE site, values decreased from 41.3 % to zero at 5–10 cm depth. The highest proportion of seeds in the seed bank (38.8 %) at the TH site was found in the surface soil layer. At the RS and WE sites, by contrast, the highest proportions were at 0–1 cm depth and amounted to 44.7 % and 41.3 %, respectively. Clearly, somewhere in the topmost centimeter seems to be the appropriate depth for germination or active seed bank of *N. procumbens*.

Table 3 Density of adult plants and seed output-seed bank ratios at the three study sites (mean ± SD). Values in the same row marked by different letters are significantly different ($P < 0.05$)

Parameter	Thumamah	Site Ras Sidr	Wadi Elnatroun
Plant density (# m^{-2})	17.3 ± 4.07^{a}	8.5 ± 2.77^{b}	10.6 ± 4.12^{b}
Seed output (# m^{-2})	512.2 ± 37.50^{a}	340.4 ± 28.80^{b}	395.4 ± 30.90^{c}
Seed bank (# m^{-2})	346.3 ± 28.10^{a}	261.8 ± 20.60^{b}	324.8 ± 35.20^{a}
Ratio	1.48 ± 0.35^{a}	1.30 ± 0.28^{a}	1.22 ± 0.26^{b}

Seed Output to Seed Bank Ratio

The density of adult individuals was almost twice as high at the TH site as at the two other sites (Table 3). Similarly, amounts of seed output and the seed bank were higher at the TH site than at the RS and WE sites. The total seed output-seed bank ratio shows a significantly lower value at the WE site than at the TH and RS sites where values exceeded unity at all three locations.

Effect of Burial Depth

Seedling emergence decreased with depth and was completely inhibited at 4 cm (Fig. 5, Table 4). Diaspores buried at the depth of 0.5 cm achieved the highest seedling emergence with the average value of 61.4±6.53 %; this gradually decreased to 1.6±1.0 % at 3 cm depth. Seedling emergence from diaspores at the soil surface was maintained at low values and averaged 6.8±3.2 % (mean ± SD). Some seedling mortality prior to emergence at the soil surface was observed in buried diaspores at depths below 2 cm.

Effect of the "Safe Side"

Seedling emergence was highly sensitive to the position of diaspores in the soil. Significant differences occurred among diaspores lying in different positions (Fig. 6, Table 5). The highest seedling emergence values of 61.4±6.5 % was reached by diaspores placed on the abaxial or "safe *side*" with the spinose side directed upwards,

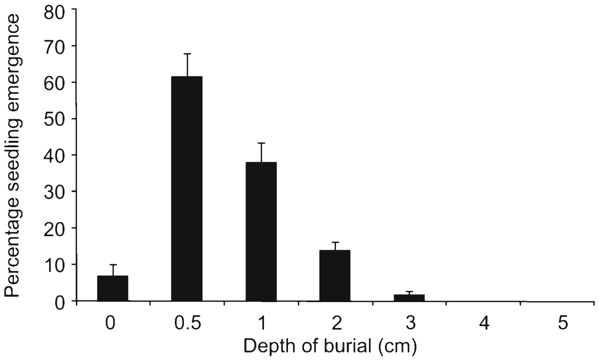

Fig. 5 Effect of fruit (diaspore) burial depth on seedling emergence of *Neurada procumbens* in the soil profile (mean ± SD)

Table 4 Table of ANOVA statistics for seedling emergence of *Neurada procumbens*. Diaspores of medium size collected at the Thumamah site were used

Source	d.f.	Mean square	*F* value	*P* value
Intercept	1	10561.829	694.205	0.000
Depth of burial	6	2784.029	182.988	0.000
Error	28	15.214		
Total	35			
Corrected Total	34			

followed by 41.6±5.6 % in the inclined diaspores with the flat, smooth side down and 28.2±5.7 % for diaspores placed vertically on the edge side. The lowest emergence, amounting to 8.2±2.9 % (mean ± SD), occurred in diaspores lying inclined with the spinose upper side down. Emergence was negligible in inverted diaspores (upside down) with the flat, smooth side facing upwards.

Seedling Establishment

The greatest extent of seedling emergence per diaspore was recorded in the large- and medium sizes, with averages of 4.0±2.0 and 3.4±2.1 (mean ± SD), respectively (range 1–7); in small diaspores average emergence was 2.4±1.5 within the range of 1 to 5 (Table 6, Appendix 3).

The dry mass of three-week-old seedlings ranged from 11.8±1.7 mg in large diaspores to 7.0±1.4 mg in small ones (mean ± SD), seedlings from medium-sized diaspores having intermediate values. Seedling dry mass was reduced, with increased numbers of seedlings per diaspore.

As regards root and shoot lengths, the root-shoot ratio exceeded the twenties and average values ranged from 22.8 in small- to 30.3 in large-diaspore seedlings, with an intermediate ratio, in medium-sized diaspore seedlings. The root length may exceed 10 cm before shoot

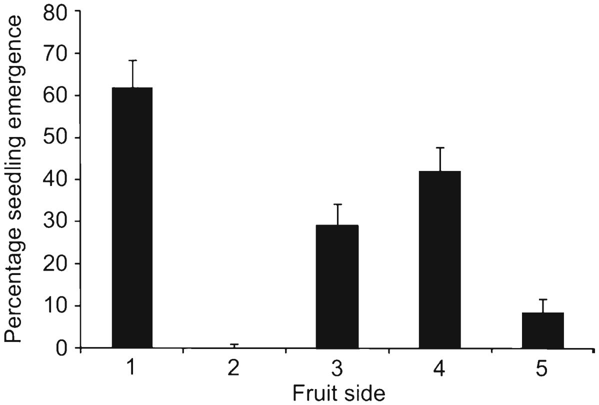

Fig. 6 Effect of fruit (diaspore) position on seedling emergence of *Neurada procumbens* (mean ± SD). (1) diaspores lying horizontally on the flat smooth lower side; (2) inverted position with diaspores lying horizontally on the spinose upper side; (3) vertical position with diaspores positioned vertically on their side; (4) inclined position with diaspores positioned inclined at different angles with their flat, smooth side upwards; and (5) inclined position with diaspores positioned inclined with their spinose side upwards

Table 5 Table of ANOVA statistics for explaining seedling emergence of *Neurada procumbens*. Diaspores of medium size collected at the Thumamah site were used

Source	d.f.	Mean square	*F* value	*P* value
Intercept	1	19712.160	703.503	0.000
Fruit side	4	3071.860	109.631	0.000
Error	20	28.020		
Total	25			
Corrected Total	24			

emergence above the soil surface. The number of emerging seedlings, seedling dry mass, root-shoot ratio and leaf area all decreased with decreasing diaspore size (Table 6).

Total leaf area was significantly greater in seedlings emerged from large diaspores than small ones (Table 6), with average values of 19.2±1.9 mm^2 and 16.0±2.1 mm^2 (mean ± SD), respectively. Seedlings from medium-sized diaspores had an intermediate leaf area.

Discussion

Successful establishment of *Neurada procumbens* in frequently disturbed desert habitats depends on the physical structure of the soil as well as on the interaction between diaspore "safe *sites*" and the orientation of their placement in the soil. This supports our hypothesis that for successful seedling establishment, diaspores should be lying on their "safe *side*".

Different aspects of soil variability may influence the dynamics of *N. procumbens* seeds and diaspores. The coarse, textured sandy soil with low organic matter content at all three locations provides an adequate "safe *site*" for *Neurada* diaspores. Coarse-textured soils in general increase the variation of diaspore burial depth in the soil profile; they also hold water at lower surface tension and provide more available water during seed germination and seedling establishment (Foth and Turk 1972; Bowers and Lowe 1986). The extremes of moisture and temperature which are usually encountered in desert environments make the germination of surface-lying seeds hazardous.

The three populations of *N. procumbens* showed site-to-site differences in seed bank germination, vertical distribution patterns and seed output. All three locations are ruderal and experience regular disturbance regimes. Disturbances in general are well known to be a major driver of seed germination and seed bank biology (Bazzaz 1984; Reichman 1984). Despite this site-to-site variation, only non-significant differences

Table 6 Seedling emergence per single diaspore and seedling growth characteristics for large, medium-sized and small diaspores (mean ± SD)

Diaspore size	Seedling emergence (# per fruit)	Seedling dry weight (mg)	Root length (cm)	Shoot height (cm)	Root-shoot ratio	Leaf area (mm^2)
Large	4.0±1.8	11.8±1.72	16.2±1.79	0.54±0.06	30.3±5.6	19.2±1.9
Medium	3.4±1.6	9.4±2.06	13.0±1.87	0.50±0.07	26.1±1.3	17.8±1.7
Small	2.4±1.5	7.0±1.41	10.8±1.10	0.48±0.08	22.8±2.6	16.0±2.1

were found among populations in experimental studies on seed germination and seedling establishment.

Disturbances exert major pressure in ruderal aridland habitats. Diaspores are subject to ongoing sand loss and accretion, mediated by wind as well as activity of small mammals, grazers and humans, all of which may bury seeds at different depths in soil. The orientation or position and depth of burial of diaspores in soil are variable and clearly affect seed germination and seedling establishment. Shallow burial seems advantageous while deep burial can prevent seedling emergence caused by pre-emergence mortality. The deep burial of diaspores results in cessation of germination and seedling growth because of a lack of oxygen and light (Zhang and Maun 1990; Vleeshouwers 1997; Li et al. 2006, 2007; Ohadi et al. 2011).

Vertical distribution of *N. procumbens* diaspores and differences in diaspore size suggest the presence of a persistent seed bank. Seed germination and seedling establishment appear to be a product of burial depth, diaspore size and "safe *side*" orientation in soil. Previous reports show that differential germination of seeds positioned on different sides at different soil depths can help ensure a more persistent seed bank. It could therefore be considered as an adaptation to unreliable rainfall patterns (see too Guo et al. 1998, 1999; Gutterman 2000).

Vertical distribution of propagules in the seed bank may offer an array of "safe *sites*", interacting with asymmetrical diaspore morphology in *N. procumbens*. The disturbance-related vertical distribution of diaspores in space and time makes conditions become more or less favorable for germination, and in some species may affect seed dormancy (Osem et al. 2006; Olvera-Carrillo et al. 2009). Vertical distribution of diaspores in soil suggests the existence of both transient and more persistent components of the seed bank. Diaspores lying no deeper than 0.5 cm from the soil surface constitute a more transient component, capable of immediate germination, and few of these remain viable for more than a year. Alternatively, most of the diaspores lying at depths below 0.5 cm in the soil profile constitute the more persistent component which may remain viable for several years depending on soil disturbance (see Thompson and Grime 1979).

A persistent seed bank likely remains in a state of dormancy while buried deep in the soil. Secondary dormancy may be induced by burial, which is often the case for species with long-lived seeds (Bonis and Le Part 1994; Baskin and Baskin 1998). This dormancy can be broken and germination promoted by the disturbance which brought the buried seeds to the surface. Pre-emergence mortality may result from cessation of seedling growth prior to reaching the surface.

Diaspores of *N. procumbens* buried at shallow depths germinated at a higher rate than diaspores lying on the surface, probably because burial provides a moister micro-environment, preventing seeds and seedlings from drying out. However, excessively deep burial may diminish seed germination and prevent emergence above ground. This is indicated by dead seedlings below the soil surface. In general, we observed that as the depth of burial increased, there was a significant decrease in both seed germination and seedling emergence. The interaction among diaspore size, depth of burial and "safe *side*" positioning suggests that diaspore and soil traits play a complex role in seed bank germinability (see too Garcia-Fayos and Gasque 2006).

Greatest emergence was obtained when diaspores were positioned on the side, ensuring maximum contact between the soil substrate and the scar of seed attachment. This position is most favorable for water uptake and subsequent germination and seedling emergence (Sheldon 1974; DeFalco et al. 2009). Diaspores set on other sides and in different orientations had significantly decreased emergence. Clearly, the occurrence of diaspores in an appropriate microsite or “safe *site*” does not ensure successful seedling emergence and establishment. Diaspores also have to be positioned in the most suitable orientation.

Conclusions

Burial of *Neurada procumbens* diaspores at different soil depths and in different orientations interacts with diaspore size variability to influence germination and seedling establishment in disturbed arid regions. Disturbances move diaspores up and down the soil profile, resulting in a bank of diaspores positioned on different sides and occupying various micro-sites. The structured vertical distribution of diaspores in the soil profile shifts the seed bank from a shorter-lived, more transient one to one that is more persistent (longer-lived).

Acknowledgments The authors are grateful to the Dean of Research at King Saud University for funding the work through research project No. RGP-VPP-175. We thank Dr. H. A. El-Adawy, Menofiya University (Sadat City), for helping us with the statistical analysis. We are grateful to the two anonymous reviewers for their helpful suggestions about the manuscript.

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Received: 21 August 2012 / Revised: 26 August 2013 / Accepted: 27 August 2013

Appendix 1

Table 7 Table of ANOVA statistics for differences in seed output in *Neurada procumbens*

Source of variation	d.f.	Mean Square	*F* value	*P* value
Site	2	12828.467	39.1536	0.0000
Error	12	327.644		
Diaspore size	2	87968.067	49.063	0.0000
Site*Size	4	7990.533	4.4566	0.0078
Error	24	1792.961		
Total	44			

Appendix 2

Table 8 Table of ANOVA statistics for differences in the soil seed bank of *Neurada procumbens*

Source of variation	d.f.	Mean Square	*F* value	*P* value
Site	2	625.098	45.9106	0.0000
Error	12	13.616		
Burial Depth	4	11663.351	650.3267	0.0000
Site*Depth	8	1032.664	57.5794	0.0000
Diaspore Size	2	4014.671	223.8506	0.0000
Site*Size	4	279.378	15.5776	0.0000
Depth*Size	8	1055.904	58.8753	0.0000
Site*Depth*Size	16	261.728	14.5935	0.0000
Error	168	17.935		
Total	224			

Appendix 3

Table 9 Table of ANOVA statistics for various seedling growth criteria in *Neurada procumbens*

Dependent variable / Source	d.f.	Mean Square	*F* value	*P* value
Seedling emergence:				
Intercept	1	160.067	36.656	0.000
Diaspore size	2	3.267	0.748	0.494
Error	12	4.367		
Total	15			
Corrected Total	14			
Seedling dry weight:				
Intercept	1	1325.400	345.757	0.000
Diaspore size	2	28.800	7.513	0.008*
Error	12	3.833		
Total	15			
Corrected Total	14			
Seedling root length:				
Intercept	1	2666.667	1012.658	0.000
Diaspore size	2	36.867	14.000	0.001*
Error	12	2.633		
Total	15			
Corrected Total	14			
Seedling shoot height:				
Intercept	1	3.851	770.133	0.000
Diaspore size	2	0.005	0.933	0.420
Error	12	0.005		
Total	15			
Corrected Total	14			
Seedling root-shoot ratio:				
Intercept	1	10422.744	1060.299	0.000
Diaspore size	2	69.998	7.121	0.009*
Error	12	9.830		
Total	15			
Corrected Total	14			
Seedling leaf area:				
Intercept	1	4681.667	1088.760	0.000
Diaspore size	2	12.867	2.992	0.088
Error	12	4.300		
Total	15			
Corrected Total	14			