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Subterranean and aerial seed production of southern threecornerjack (*Emex australis*)

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Research Article

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Abstract

Southern threecornerjack (*Emex australis* Steinh.) produces both aerial seeds on the branches and subterranean seeds at the base of the plant crown approximately 1 to 2 cm underground. Prior research indicated that seeds buried at a depth of 1 cm have a much higher probability of germinating in the following season compared with seeds on the soil surface. The current research investigated the number of subterranean seeds produced per plant, timing of subterranean seed production, and germination requirements of subterranean seeds compared with aerial seeds. This research demonstrated that *E. australis* plants from multiple populations produced subterranean seeds beginning at the 4-leaf development stage, before the production of aerial seeds. Individual plants produced a maximum of 30 subterranean seeds. Germination of subterranean and aerial seeds following afterripening over the summer to autumn (December to May) fallow was similar, demonstrating that the different seed types had no differences in dormancy. Further, the subterranean seeds did not have the light requirement for germination observed in aerial seeds. Subterranean seeds had 22.2% and 24.3% germination in light and dark conditions, respectively, compared with aerial seeds, which had 26.9% germination in light conditions and 12.3% germination in the dark. Production of subterranean seeds was low, given that a mature *E. australis* plant may produce more than 1,100 aerial seeds, but the subterranean seeds formed earlier in plant development. Recommendations for postemergence herbicides are to spray *E. australis* at the 2- to 4-leaf stage of growth, yet some plants may have already produced mature subterranean seeds by this stage. Further research is required to determine the impact of subterranean seeds on population ecology.

Introduction

Southern threecornerjack (*Emex australis* Steinh.; syn.: *Rumex hypogaeus* T.M.Schust. & Reveal; family Polygonaceae) is a native of South Africa and a widespread winter annual weed in Australia, New Zealand, and the United States (CABI 2020; Llewellyn et al. 2016; Schuster et al. 2015). The total expenditure on control of *E. australis* and yield revenue loss from surviving plants make it the fifth most costly weed in the Western Australian wheatbelt (grain cropping and pasture region) and the 16th most costly weed nationally (Llewellyn et al. 2016). In Western Australia, a density of 32 *E. australis* plants m⁻² reduced wheat (*Triticum aestivum* L.) yield by 10% (Dhammu et al. 2020). Conversely, Borger et al. (2020b) did not see a reduction in wheat yield from *E. australis* at a density of 6 to 57 plants m⁻². In Faisalabad, Pakistan, a density of 32 *E. australis* plants m⁻² reduced wheat yield by 44% to 62% (Javaid et al. 2016). Aside from direct crop competition, the seeds of *E. australis* also cause economic loss. First, seeds contaminate grain at harvest, as these prostrate plants can vine up into the crop canopy (Michael et al. 2010). Second, the seeds are formed within a hard, wooden achene with three spines and can cause lameness in livestock (Gilbey et al. 1998).

Avoiding economic loss depends on identifying weed management strategies to minimize *E. australis* growth and seed production. This issue is complicated in *E. australis*, because seeds (achenes) form first on the lower sections of the crown of the plant (subterranean seeds; Gilbey et al. 1998). These subterranean seeds sit at a depth of approximately 1 to 2 cm below the soil surface. More seeds develop on the upper sections of the crown and leaf axils along the stems (aerial seeds; Weiss 1981). The plants are monoecious, but it is unknown if either subterranean or aerial seeds are most commonly produced by autogamy or allogamy (Gilbey et al. 1998). However, there is very little genetic diversity in *E. australis*, and Australian populations remain genetically similar to the source populations in South Africa (Panetta and Randall 1993). Aerial seeds easily detach as the parent senesces and degrades. They are triangular in cross section, with spines arranged so that one spine always points upward to increase the chance of dispersal via vehicles or livestock, although seeds are also distributed by water movement or in hay or grain (Gilbey et al. 1998; Lemerle 1996; Michael et al. 2010; Figure 1). The subterranean seeds remain attached to the parent (below the soil surface) after senescence and are much less

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Figure 1. *Emex australis* seeds have dimorphic morphology: aerial seeds (left) have radial symmetry, and subterranean seeds (right) have bilateral symmetry and shorter spines.

likely to disperse, although in an agricultural system, the tines on a crop seeder may drag the seeds through the soil horizontally (Gilbey et al. 1998; Powles and Bowran 2000).

In favorable conditions, *E. australis* growth is indeterminate, with continuous stem and seed production (Panetta 1990; Weiss 1981). Individual plants in favorable conditions can produce more than 1,100 aerial seeds, and a population growing uncontrolled the field for 1 yr produced a seedbank with up to 4,600 seeds m^{-2} (Weiss 1978, 1981). For aerial seeds, dormancy and germination/emergence in the field has been assessed. Seeds collected in southern Australia and tested in controlled conditions were initially 100% dormant and required a 2- to 6-mo period of afterripening at high temperatures over the summer fallow. Following afterripening, 80% to 95% of seeds would germinate following exposure to sufficient moisture in the autumn/winter growing season (Panetta and Randall 1993). In the absence of favorable growing conditions, the majority of seeds (60% to 90%) cycled dormancy, with germination being possible at winter temperatures but not at the higher summer temperatures (Cheam 1996; Panetta and Randall 1993). However, in field conditions, the proportion of seeds that germinate is low, with 17% reported by Cheam (1996), 18% by Panetta and Randall (1993), and 37% by Weiss (1981). Low field emergence results, because emergence is dependent on both burial and dormancy cycles (Cheam 1996; Panetta and Randall 1993). Emergence was favored by shallow burial, with emergence of 23.5%, 53.9%, 28.1%, 3.3%, and 0% for seeds at depths of 0 (i.e., on the soil surface), 1, 5, 10, and 15 cm, respectively, in the field over 4 yr (Cheam 1996). However, in the zero-tillage cropping system common to Western Australia, where growers aim to disturb less than 5% to 10% of the soil surface, aerial seeds shed from the mature plant may remain on the surface (Cheam 1996; Gilbey et al. 1998; Powles and Bowran 2000). Dormancy and germination have not been assessed for subterranean seed. Conditions during seed maturation (photoperiod, temperature, and light quality) can impact dormancy of individual seeds shedding from a single plant (Finch-Savage and Leubner-Metzger 2006). Clearly subterranean seeds and aerial seeds are subject to different environmental conditions during maturation. Because subterranean seeds develop at an ideal depth of 1 to 2 cm to maximize emergence, it is possible that, following afterripening, these seeds have less initial dormancy than aerial seeds.

Studies on the seed ecology of this species are limited to aerial seeds (Gilbey 1990; Javaid and Tanveer 2014). Previous research does not specify characteristics of subterranean seeds. Aerial seeds can develop 6 wk after emergence (Gilbey et al. 1998; Weiss 1981). Timing of subterranean seed development has not been investigated, although it is generally assumed that they form before aerial seeds (Gilbey et al. 1998; Weiss 1981). Germination of aerial seeds is stimulated by light availability, although germination may also occur in the dark (Javaid and Tanveer 2014). As subterranean seeds form underground and are not exposed to light in an undisturbed system, it is possible these seeds do not have increased germination following exposure to light. Germination of aerial seeds is also favored by scarification, particularly in freshly harvested seed (before afterripening; Hagon and Simmons 1978). However, aerial seeds on the soil surface, with one spine always pointing upward, have greater capacity for physical damage of the seed coat via livestock or abrasion against the soil (Gilbey et al. 1998). Scarification has not been investigated for subterranean seeds, but in undisturbed conditions, subterranean seeds are less likely to be damaged and may not be affected by scarification.

This research aimed to investigate both the number of subterranean seeds produced and the timing of seed production and to compare the germination requirements of subterranean and aerial seeds. The first hypothesis was that all *E. australis* populations would produce subterranean seeds before aerial seeds. This was tested by monitoring seed production of a single population in two different locations (field or screen house) in a 2018 experiment and of multiple populations in a single location (screen house) in a 2020 experiment. The current research did not seek to separate the environmental and genetic factors affecting seed production in *E. australis*, but rather to test the hypothesis for a range of populations in different environments. The second hypothesis was that subterranean seeds would have higher germination (less initial dormancy) than aerial seeds. This hypothesis was tested by assessing germination of aerial and subterranean seed harvested during the 2018 and 2020 experiments. The third hypothesis was that the germination of aerial seeds would be stimulated by light and scarification to a greater extent than that of subterranean seeds. This was tested through germination experiments using seed harvested during the 2020 experiment.

Materials and Methods

Timing of Seed Production in the Field or Screen House in 2018 and Screen House in 2020

In December 2017, mature, aerial *E. australis* seeds were manually collected from a population in an arable field at Wongan Hills, WA (30.8809°S, 116.7262°E). A Wongan Hills population was selected because the 2018 experiments aimed to compare growth of a single population in the field at Wongan Hills or a screen house. We did not desire to introduce a new ecotype of *E. australis* to the field site at Wongan Hills. In January 2020, aerial seeds were manually collected from six populations in arable fields in Grass Valley (31.6339°S, 116.7879°E, average rainfall of 431 mm), Wongan Hills (362 mm), Northam (31.6698°S, 116.6371°E, 427 mm), York (31.9245°S, 116.9177°E, 451 mm), Moora (30.5776°S, 116.1592°E, 461 mm), and Bolgart (31.3865°S, 116.5075°E, 366 mm) in Western Australia. The 2020 screen house experiment aimed to compare growth of multiple populations in a single environment. For both collection times, seeds were stored in an open glasshouse until the following April to allow afterripening (Weiss 1981).

Table 1. Climatic conditions for the 2018 field experiment at Wongan Hills, WA, and the 2018 and 2020 screen house experiments at Northam, WA, including total monthly rainfall (mm), and average maximum and minimum temperature (C).

| Experiment ^a | Climatic data | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|----------------------------|---------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 2018 field: Wongan Hills | Rainfall | 44 | 5 | 3 | 0 | 32 | 51 | 80 | 91 | 9 | 31 | 10 | 4 |
| | Max. temp. | 34 | 33 | 32 | 27 | 23 | 18 | 17 | 17 | 21 | 25 | 29 | 34 |
| | Min. temp. | 17 | 18 | 17 | 12 | 9 | 7 | 7 | 6 | 6 | 11 | 12 | 16 |
| 2018 screen house: Northam | Rainfall | 78 | 4 | 1 | 4 | 35 | 49 | 119 | 95 | 7 | 45 | 3 | 3 |
| | Max. temp. | 33 | 33 | 32 | 27 | 24 | 18 | 18 | 17 | 21 | 25 | 29 | 33 |
| | Min. temp. | 16 | 16 | 15 | 11 | 7 | 6 | 6 | 5 | 6 | 11 | 12 | 16 |
| 2020 screen house: Northam | Rainfall | 0 | 42 | 10 | 8 | 38 | 42 | 29 | 45 | 23 | 1 | 45 | 4 |
| | Max. temp. | 34 | 34 | 31 | 28 | 22 | 21 | 19 | 18 | 22 | 27 | 27 | 34 |
| | Min. temp. | 17 | 19 | 16 | 14 | 7 | 7 | 5 | 6 | 8 | 10 | 12 | 16 |

^aNote that screen house rainfall was supplemented with irrigation.

Table 2. The harvest date of *Emex australis* plants from the field or screen house experiments (and the number of plants in each harvest), leaf number (range), growth stage, and percent of plants harvested at each date with no seeds, only subterranean seeds, or both aerial and subterranean seeds.

| Experiment | Harvest date (number of plants) | Leaf number | Growth stage | Percent of plants harvested with ^a | | |
|-------------------|---------------------------------|-------------|---------------------------|---|--------------------|-------------------------------|
| | | | | No seeds | Subterranean seeds | Subterranean and aerial seeds |
| 2018 field | 8/24/2018 (10) | 4–13 | Flowering–seed production | 20 | 80 | 0 |
| | 9/14/2018 (2) | 11–37 | Seed production | 0 | 0 | 100 |
| | 9/20/2018 (18) | 4–35 | Flowering–seed production | 0 | 22 | 78 |
| | 10/19/2018 (7) | 5–94 | Seed production | 0 | 29 | 71 |
| | 11/12/2018 (30) | 21–342 | Seed production | 0 | 0 | 100 |
| 2018 screen house | 6/29/2018 (10) | 2 | Vegetative | 100 | 0 | 0 |
| | 7/17/2018 (10) | 4 | Vegetative | 100 | 0 | 0 |
| | 8/9/2018 (10) | 6 | Vegetative–flowering | 100 | 0 | 0 |
| | 9/6/2018 (14) | 8–37 | Seed production | 0 | 21 | 79 |
| | 9/19/2018 (10) | 4–37 | Seed production | 10 | 0 | 90 |
| 2020 screen house | 4/17/2020 (33) | 16–36 | Flowering–seed production | 0 | 100 | 0 |
| | 4/28/2020 (52) | 4–29 | production | 0 | 83 | 17 |
| | 5/8/2020 (34) | 10–26 | | 0 | 21 | 79 |
| | 5/13/2020 (14) | 10–28 | | 0 | 50 | 50 |
| | 5/20/2020 (6) | 10–12 | | 0 | 100 | 0 |
| | 5/29/2020 (16) | 10–18 | | 0 | 50 | 50 |

^aNote that there were no plants harvested with only aerial seeds and no subterranean seeds.

The seeds collected from the Wongan Hills population were used to establish a field experiment at the Department of Primary Industries and Regional Development (DPIRD) Wongan Hills Research Station in 2018 (referred to as the 2018 field experiment, 30.8438°S, 116.7457°E). The experimental site was yellow orthic acidic Tenosol soil (Isbell 2016), with pH (CaCl₂) of 5.8 and organic carbon of 0.05% in the top 0 to 10 cm, and winter-dominant rainfall (Table 1). The nonselective herbicide glyphosate at 1,080 g ai ha⁻¹ (Roundup® PowerMax®, 540 g ai L⁻¹, AC, Nutrien Ag Solutions, 16 Yilgarn Ave, Northam, WA 6401, Australia) plus carfentrazone-ethyl at 10 g ai ha⁻¹ (Hammer® 400EC, 400 g ai L⁻¹, EC, Nutrien Ag Solutions, 16 Yilgarn Ave, Northam, WA 6401, Australia) was applied to control other weed species in the plots before seeding on May 29, 2018. *Emex australis* seeds were spread by hand at a rate of 100 seeds m⁻² in three plots of 3 m by 30 m and incorporated via shallow cultivation into the top 4 cm of the soil. *Emex australis* seedlings emerged from August 2018. Non-target weed species were manually removed (no other preemergence or postemergence herbicide was applied to the site). The site was visited approximately every week, and plants were harvested at varying growth stages (Table 2). Note that plants were not harvested every week, as plants at the appropriate growth stage

were not always available. Plants selected for harvest had entered the reproductive stage, as seeds cannot form before anthesis. The spiny female flowers are sessile, in axillary clusters that form first on the plant crown, and are easily visible before harvest (Gilbey et al. 1998).

Two screen house experiments were conducted at the DPIRD Northam office (referred to as 2018 or 2020 screen house experiments; 31.6509°S, 116.6978°E). The 2018 screen house experiment was established on May 21, 2018, using seeds from the Wongan Hills population. The 2020 screen house experiment was established on February 14, 2020, using seeds from all six populations. Therefore, the 2020 plants were exposed to higher temperatures than the 2018 plants (Table 1), although rainfall was supplemented with irrigation for both experiments to ensure healthy growth. For each experiment, 10 pots of 53 cm long by 12 cm wide by 12 cm depth (per population) were filled to within 2 cm of the top with 5 kg of potting mix per pot (where potting mix had a pH [CaCl₂] of 6.9 and organic carbon of 15.8%). Twenty-five seeds were planted per pot at a depth of 1 cm. Following planting, the experiments were visited every 2 d to record seedling emergence. In the 2018 screen house experiment, plants were harvested on four dates, from the 2-leaf stage to the branching stage (Table 2). In the

2020 screen house experiment, plants were harvested on seven dates, from the 4-leaf stage to the branching stage (i.e., from anthesis onward). In both experiments, approximately a fifth of seeds emerged. In 2018, all plants were harvested. In 2020, about half of the plants were harvested for the experiment. Harvest was staggered for each pot (i.e., some plants removed from each pot at each harvest time) to avoid competition affecting plant development. Further, excess (unused) seedlings were harvested and discarded from the pots in the 2020 experiment.

Following harvest in each experiment, leaf number was recorded, and aerial and subterranean seeds were manually removed and counted. Leaf number was selected as an assessment of plant growth stage/size, because this is the accepted method of assessing the plant development stage of dicotyledonous species until the development of side shoots (Hess et al. 1997). Assessment of leaf number also offered a continuous variate for comparison with number of seeds, which can easily be assessed by land managers in the field. In the 2020 screen house experiment, plant roots were excavated and seeds manually removed in a dark room with no natural light, with seeds removed by touch, and then subterranean seeds were stored in dark conditions. *Emex australis* growth is indeterminate, and the aim of the experiments was not to determine maximum possible plant size. For all experiments, harvest continued up to the stage where maximum subterranean seed production was determined. This was done by examining the harvest data directly after each harvest event, and harvest ceased when subterranean seed number stopped increasing, even though leaf number and aerial seed number continued to increase. For all experiments, there was broad variation in timing of emergence and rate of growth, so different numbers of plants were harvested at each date (Table 2). Totals for plants harvested during the experiments were 67 plants from the 2018 field experiment, 55 plants from the 2018 screen house experiment, and 149 plants from the 2020 screen house experiment.

Regression analysis was used to compare leaf number with total seed production, subterranean seed production, and aerial seed production in each experiment. For the 2018 field or screen house experiments, a linear model was used. However, for the 2018 field population, linear correlation was very low, so a linear model with an exponential function (origin constrained) was applied. For the 2020 screen house experiment, a linear model with groups was used, with population as the grouping factor. Accumulated ANOVA was used to determine whether there was a significant effect of grouping by population. For all data sets, residual plots were used to confirm the normal distribution of the data (Genstat, 20th ed., VSN International, Hemel Hempstead, UK; www.vsn.co.uk). The aerial seed data from the 2020 screen house experiment did not have a normal distribution of residuals, because there were too many zero values (i.e., plants with no aerial seeds). The higher number of zero values also made alternative analyses impractical, and transformation of the data did not normalize the distribution of residuals. Therefore analysis was not applied to this data set, although the data were still presented with the other data sets from the 2020 screen house experiment.

Germinability and Viability of Seeds Harvested from the 2018 or 2020 Experiments

Following harvest of the subterranean and aerial seeds from each experiment, all seeds were afterripened in an open glasshouse over the following summer (December to February). As stated, subterranean seeds from the pots in 2020 were harvested in dark conditions

and then stored in dark conditions for afterripening. Conversely, aerial seeds were exposed to light during afterripening. This system was used to mimic the field conditions over the summer fallow, where subterranean seeds remain underground and aerial seeds are shed onto the soil surface. Following afterripening, seeds were bulked according to seed type (subterranean or aerial) for the 2018 screen house and field experiments and according to seed type and population for the 2020 screen house experiment.

For seed harvested in 2018, an experiment was established to investigate two factors; seed type and location in which the parental plants were grown (field or screen house). For seed harvested in 2020, an experiment was established to investigate four factors: seed type, population (Grass Valley, Wongan Hills, York, Moora, and Bolgart), scarification (with or without scarification), and light requirements (exposure to natural light or no light) for germination. Note that the population from Northam in the 2020 experiment was not included in this experiment due to insufficient seed production. Both experiments were arranged in a completely randomized design, with three replications. Three replications were selected because germination was not expected to have high variation within populations.

The treatments in each experiment were established by placing 25 *E. australis* seeds in 9-cm petri dishes containing two layers of Whatman No. 1 filter paper (Australian Scientific Pty Ltd, 11 McDougall St, Kotara, NSW 2289, Australia). Petri dishes were moistened with 10 ml of distilled water containing 250 ppm iprodione to control fungi, sealed with parafilm (Westlab, 4 Cargo Way, Mitchell Park, VIC 3355, Australia, <https://westlab.com.au>), and placed in germination cabinets at 20/10 C (optimal germination temperature; Javaid and Tanveer 2014). Petri dishes were exposed to a 12-h photoperiod illuminated by fluorescent lamps at a light intensity of 85 $\mu\text{mol m}^{-2} \text{s}^{-1}$. For the 2020 seeds, to test scarification, seeds were rubbed between two sheets of sandpaper to remove spines. To test light requirements for germination, petri dishes were either wrapped in two layers of aluminum foil (germination in dark conditions) or left unwrapped (germination in light). As stated, for subterranean seeds in 2020, the initial harvest and afterripening was conducted in a darkened room. Likewise, scarification, placing seeds in petri dishes, and wrapping petri dishes in aluminum foil was conducted by touch in a darkened room. Therefore, seeds tested for germination in dark conditions had never been exposed to natural light. This was to mimic field conditions, where subterranean seeds develop, afterripen, and subsequently germinate underground.

In each experiment, the number of germinated seeds was counted over 4 wk, where germination was defined as the emergence of a radicle of at least 1 cm. Ungerminated seeds were retrieved and exposed to tetrazolium chloride for 24 h. Embryos were then manually extracted, with the woody seed coats crushed and pulled apart with pliers to test viability (Steadman et al. 2003). While this method of extraction usually damaged the embryo, it was still possible to determine whether the embryo was fully purple or had any white/pink sections that would indicate unviability.

An ANOVA was used to assess data. For the 2018 experiment, seed type (aerial or subterranean) and location (2018 field or screen house seeds) were factors. For the 2020 experiment, seed type, scarification, light, and population were factors. The response variables for both experiments were percent germination or percent viability per petri dish, where viable seeds were germinated seeds plus ungerminated viable seeds identified by the tetrazolium chloride assay. Replication was the blocking factor. Means were separated by LSD, and residual

plots were used to confirm the normal distribution of the data (Genstat, 20th ed.).

Results and Discussion

Timing of Seed Production in the Field or Screen House in 2018 or Screen House in 2020

This research confirms the first hypothesis, that plants in the field or controlled conditions produce subterranean seeds before aerial seeds. Plants were harvested with no seeds, subterranean seeds alone, or both subterranean and aerial seeds (Table 2). There were no plants in any experiment with only aerial seeds and no subterranean seeds. Managers can be confident that if aerial seeds are apparent, subterranean seeds have already developed. The size of the plants harvested in each experiment ranged from 4 to 342 leaves for 2018 field plants, 2 to 37 leaves for 2018 screen house plants, and 4 to 36 leaves for 2020 screen house plants (Table 2). In the 2018 screen house experiment, plants produced subterranean seeds at the 8-leaf stage and aerial seeds at the 10-leaf stage (Table 3). These plants had a strong correlation between leaf number and total seed production and a lower (but still highly significant) correlation between leaf number and subterranean or aerial seed number (Figure 2). Plants in the 2018 field experiment produced subterranean seeds from the 4-leaf stage, and by the 5-leaf stage, plants also produced aerial seeds (Table 3). In comparison to the 2018 screen house experiment, the field plants had a very poor linear correlation between leaf number and total seeds, subterranean seeds, or aerial seeds (with R^2 values of less than 10; data not presented). The correlation was slightly improved with an exponential relationship but still low ($R^2 = 29.3$ to 39.1 ; Figure 3). The exponential relationship indicated that these field plants were growing further vegetative biomass without increasing aerial seed production from the development stage of 50 leaves to 340 leaves. In the 2020 screen house experiment, plants produced subterranean seeds at the 4-leaf stage. For this experiment, the correlation between leaf number and subterranean seed number was similar to that of the 2018 screen house experiment (Figures 2 and 4). However, the correlation between leaf number and aerial seed number could not be analyzed, as there were so many plants with zero aerial seeds (Figure 4). As a result, total seed number and leaf number for the 2020 screen house experiment had a lower correlation than subterranean seed number and leaf number. While there were multiple populations in the 2020 screen house experiment, grouping by population did not significantly improve the regression relationship between leaf number and seed number, so the entire, ungrouped data set is presented. As discussed, there is little genetic variation between *E. australis* populations (Panetta and Randall 1993). The variation in correlation between leaf number and seed number among the three experiments highlights the variable growth and development of *E. australis* noted in prior research, which was related to environmental conditions (Weiss 1978). The plasticity in development in the current study may be related to climatic conditions, as the 2020 screen house plants were exposed to higher average temperatures than the 2018 experiments (Table 1). High temperatures may reduce seed development, as has been noted in other species (reviewed by Hampton et al. 2016). Across all experiments, plants could reach the 5- to 16-leaf stage without producing any seeds and the 13- to 37-leaf stage with only subterranean seeds and no aerial seeds (Table 3).

As well as variation in earliest subterranean seed production, there was variation in the maximum number of subterranean or

aerial seeds on an individual plant (Table 3). In the screen house in 2018 and 2020, plants reached maximum subterranean seed production at a similar stage (40 or 37 leaves per plant), and in the 2018 field experiment, plants were close to maximum subterranean seed production (12 seeds) at 49 leaves per plant (Figures 2–4). However, the multiple populations in the 2020 experiment produced up to twice as many subterranean seeds as the 2018 Wongan Hills plants in the field or screen house (Table 3). Aerial seed production in the 2018 screen house or field experiments increased (linearly or exponentially) with leaf production and reached a maximum of 47 seeds at 37 or 97 leaves (Figures 2 and 3). By comparison, some of the plants in the 2020 screen house experiment reached up to 37 leaves with 0 aerial seeds (Figure 4). As stated, plants in the screen house were only harvested until the number of subterranean seeds per plant stopped increasing. While field plants in this study were harvested with up to 345 leaves and a maximum of 47 seeds, most plants had fewer than 20 aerial seeds; all plants were actively growing at harvest, so they were unlikely to have reached their maximum potential seed production (Figure 3). As stated, *E. australis* plants in favorable conditions can produce more than 1,100 aerial seeds per plant in pot or field conditions, and growth in favorable conditions is indeterminate, but individual plants in the field growing in competition with a crop produced 0 to 450 aerial seeds (Borger et al. 2020a; Weiss 1978). The current study did not record date of emergence in the field. As a result, it was not possible to assess number of days to reach seed production. However, Weiss (1978) determined time to seed production was about 8 wk in pot or field experiments in Canberra, Australia. While his study did not distinguish between subterranean and aerial seeds, he did remove entire plants to assess seed number, and so his study probably included both types of seed. However, results from Weiss (1978) also highlighted the plasticity of *E. australis* development, with stress inducing seed set from an earlier date. Results from Borger et al. (2020a) indicated seasonal variation in percent survival to seed production of a single population of *E. australis* plants at Wongan Hills, WA, over 3 yr, and variation in maximum lifespan of plants between 133 and 179 d (or 1,826 to 2,640 growing degree days). Further research is required to determine the impact of environmental conditions and stress on phenology.

The current research did not aim to separate the environmental and genetic factors affecting *E. australis* seed production, but only to highlight that subterranean seed consistently formed at an earlier leaf stage than aerial seed, regardless of population or location in which the plants were grown. Prior research has highlighted the staggered emergence of *E. australis* cohorts, indeterminate growth, and plasticity in timing of seed production or total seed production (Borger et al. 2020a; Gilbey et al. 1998). Borger et al. (2020a) noted variable development of *E. australis*, as well as other dicotyledonous weeds like prostrate knotweed (*Polygonum aviculare* L.), annual sowthistle (*Sonchus oleraceus* L.), and puncturevine (*Tribulus terrestris* L.) in field conditions in Western Australia. This was in contrast to monocotyledonous weeds like rigid ryegrass (*Lolium rigidum* Gaudin) and rigput brome (*Bromus diandrus* Roth) in the same field, where plants in a single cohort had uniform timing of development, seed production, and senescence (Borger et al. 2020a). In glasshouse conditions, Ashworth et al. (2015) found considerable variation in flowering time for wild radish (*Raphanus raphanistrum* L.) and further determined that flowering time could be halved with five generations of selective breeding. It is clear that multiple dicotyledonous weed species experience plasticity in growth and timing of seed production. This will make

Table 3. Number of leaves on *Emex australis* plants at the earliest production of subterranean or aerial seeds, the maximum leaf number plants could reach with no subterranean seeds or no aerial seeds, and the maximum number of subterranean or aerial seeds on plants harvested from the 2018 field, 2018 screen house, or 2020 screen house experiments (with total plant number harvested in each experiment).

| Experiment (and number of harvested plants) | Leaf number at earliest production of | | Maximum leaf number of plants with no | | Maximum number of | |
|---|---------------------------------------|--------------|---------------------------------------|--------------|---------------------------|--------------|
| | Subterranean seeds | Aerial seeds | Subterranean seeds | Aerial seeds | Subterranean seeds | Aerial seeds |
| | | | leaves plant ⁻¹ | | seeds plant ⁻¹ | |
| 2018 field (67) ^a | 4 | 5 | 5 | 13 | 13 | 47 |
| 2018 screen house (55) ^a | 8 | 10 | 6 | 15 | 14 | 47 |
| 2020 screen house (149) | 4 | 10 | 16 | 37 | 30 | 26 |

^aThe 2018 experiments only used the Wongan Hills population.

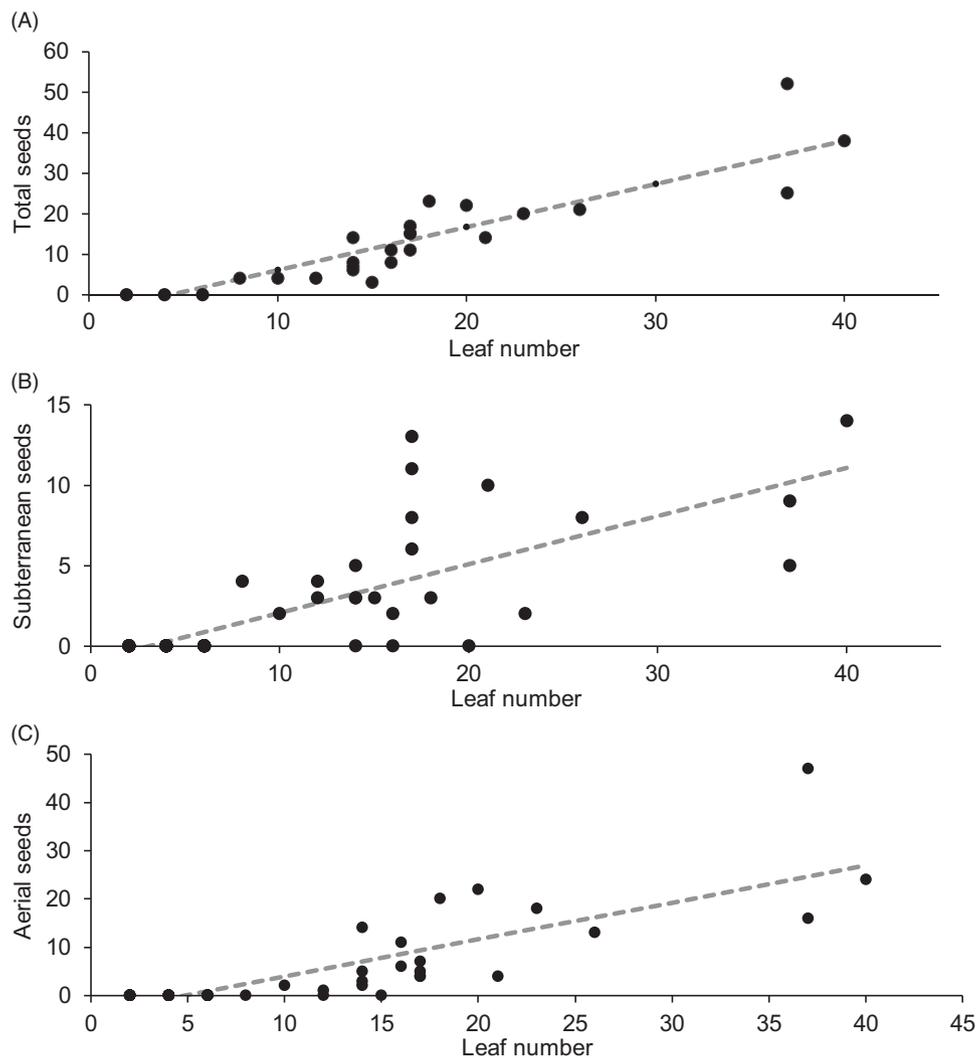


Figure 2. Regression analysis of the 2018 screen house experiment (Wongan Hills population) for the correlation between leaf number per plant and total seed production (A, $y = -4.634 + 1.0666 \cdot X$, $R^2 = 86.5$, $P < 0.001$), subterranean seed number (B, $y = -0.92 + 0.2999 \cdot X$, $R^2 = 57.6$, $P < 0.001$), or aerial seed number (C, $y = -3.71 + 0.7667 \cdot X$, $R^2 = 67.8$, $P < 0.001$).

it more difficult for managers to time weed control tactics to ensure plants are killed before seed set, unless preemergence herbicides are used.

From the current results, it is clear that the recommendation on many postemergence herbicide labels to spray *E. australis* at the 2- to 4-leaf stage will prevent aerial seed set but may be too late to completely prevent seed set in those populations producing seeds

at the 4-leaf stage (Moore and Moore 2014). However, spraying at this stage will prevent most seed set, and the viability and germinability of those early seeds (specifically those developed at the 4-leaf stage) or their potential impact on population ecology has not been investigated. There is currently no research to determine the impact of herbicides on subterranean seed development, and it may be possible to utilize preemergence or residual herbicides to

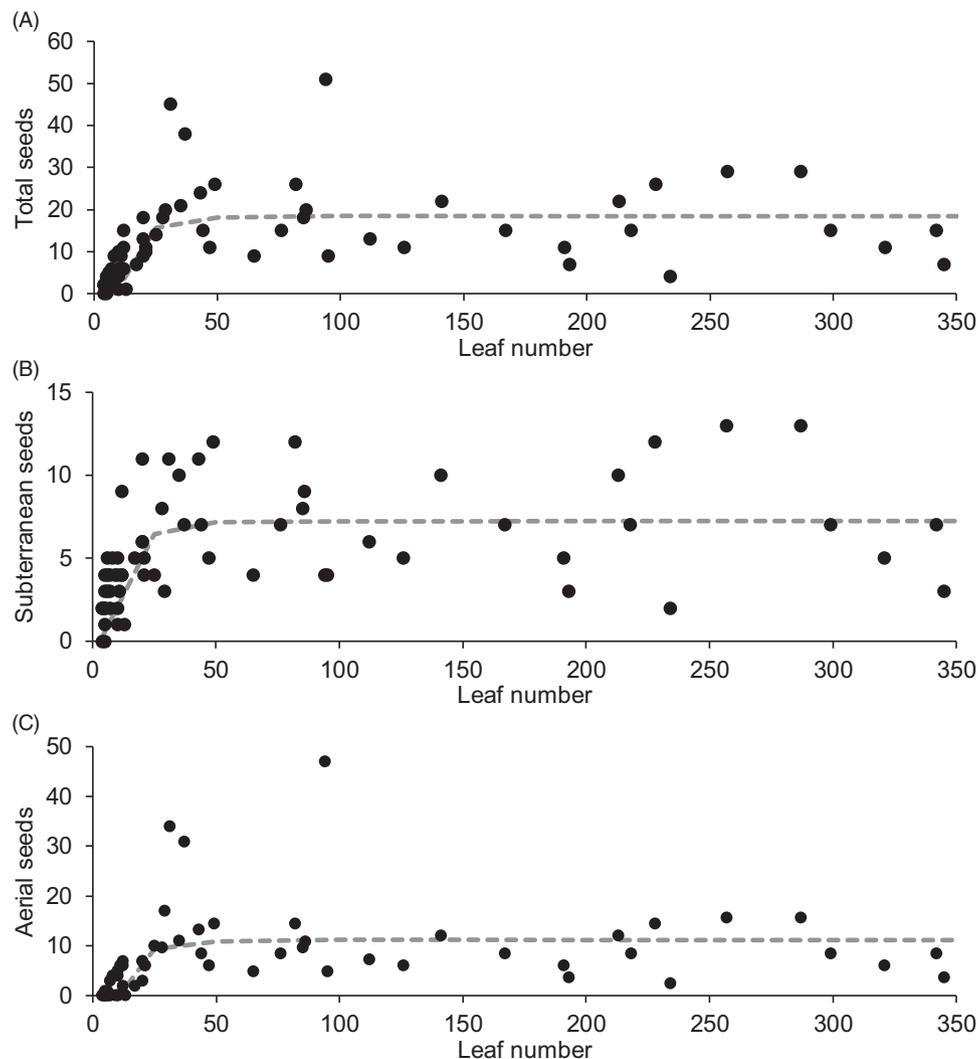


Figure 3. Regression analysis of the 2018 field experiment (Wongan Hills population) for the correlation between leaf number per plant and total seed production (A, $y = 18.4 - 26.32 \cdot 0.9129^x$, R^2 39.1, $P < 0.001$), subterranean seed number (B, $y = 7.243 - 8.14 \cdot 0.9105^x$, R^2 35.3, $P < 0.001$), or aerial seed number (C, $y = 11.15 - 18.22 \cdot 0.9136^x$, R^2 29.3, $P < 0.001$).

prevent plant establishment. Given the plasticity in *E. australis* emergence and development, as highlighted here and in prior research, it would be highly beneficial to identify herbicides that can reduce seed set and seed viability after the plants reach reproductive maturity (Gilbey et al. 1998; Weiss 1981). For example, application of glyphosate to *L. rigidum* post-anthesis (during seed production) reduced seed viability, and those seeds that retained viability had reduced seedling fitness (Steadman et al. 2006). It may be possible to identify herbicides with a similar impact on *E. australis*.

Germinability and Viability of Seeds from the 2018 or 2020 Experiments

The second hypothesis, that subterranean seeds would have higher initial germination (less dormancy) than aerial seeds, was not supported. Germination was not affected by seed type for seeds produced in the 2018 or 2020 experiments (Table 4). However, as established in the “Introduction,” optimal seed emergence in the year following seed production occurs following shallow burial (Cheam 1996). Subterranean seeds are therefore likely to have

proportionately greater initial emergence in field conditions than aerial seeds shed onto the soil surface (Cheam 1996). The action of crop sowing may bring subterranean seed to the surface or bury aerial seeds, but the no-tillage seeding systems increasingly common to the conservation agriculture systems used in Australia aim to disturb as little of the soil as possible (less than 30% of the topsoil disturbed; Busari et al. 2015). Total seed viability (germinated seeds and ungerminated viable seeds as indicated by the tetrazolium chloride test) averaged 95.7% for the 2018 experiments and 94.6% for the 2020 experiment. As for germination, viability was also not affected by seed type or the other factors in either experiment (i.e., location in 2018 experiments and population, scarification or light in 2020). This result reflects prior research, wherein different environments or stress treatments had no impact on *E. australis* seed viability (Panetta and Randall 1993; Weiss 1978).

The third hypothesis, that germination of aerial seeds would be stimulated by light and scarification to a greater extent than subterranean seeds, was partially supported. Total germination was greater in the light than the dark, but there was a significant interaction of light requirement and seed type (Table 4; Figure 5). Subterranean seeds had similar germination in light and dark

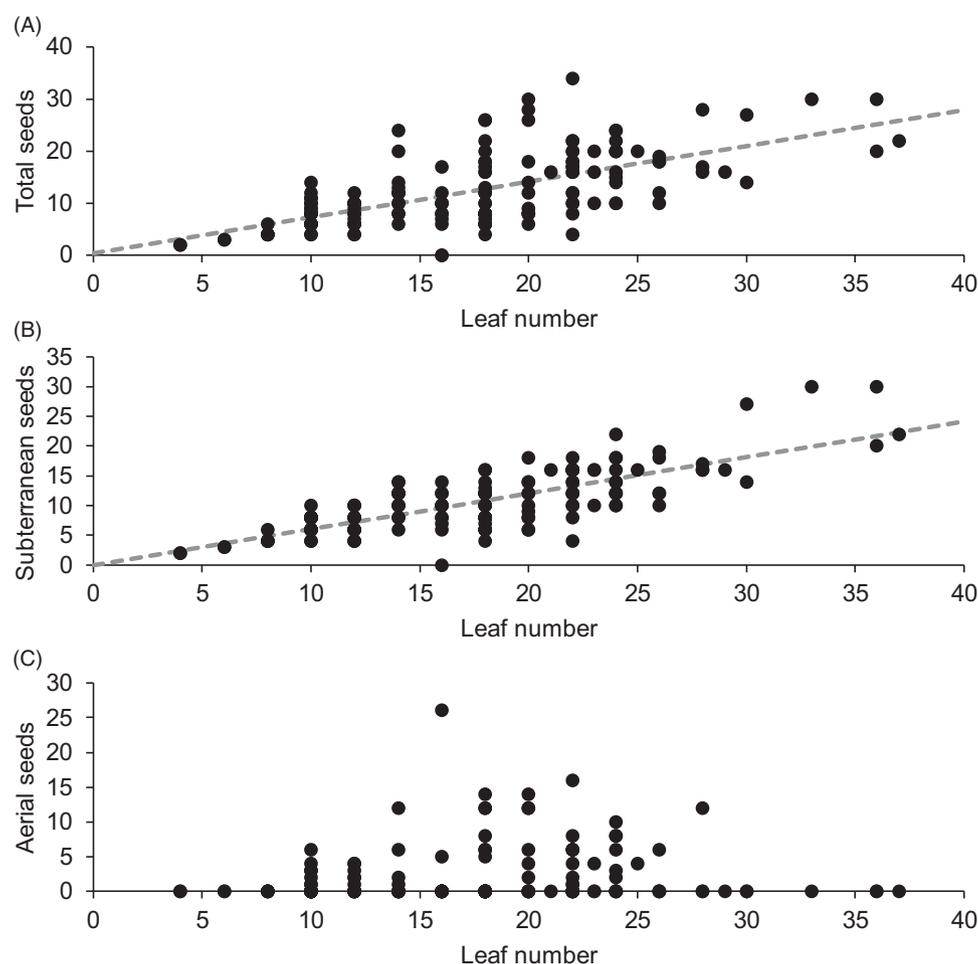


Figure 4. Regression analysis of the 2020 screen house experiment (Wongan Hills, Bolgart, Northam, Moora, Grass Valley, and York populations) for the correlation between leaf number per plant and total seed number (A, $y = 0.35 + 0.6878 \cdot X$, $R^2 = 41.2$, $P < 0.001$) or subterranean seed number (B, $y = -0.35 + 0.6047 \cdot X$, $R^2 = 58.7$, $P < 0.001$). Note that the data for aerial seed number (C) were not normally distributed, and a valid regression analysis was not performed.

Table 4. Germination percentage (from three replications of 25 seeds) of seed produced in 2018 experiments, comparing seed type and location of seed production (Wongan Hills field site or screen house), and 2020 experiment, comparing seed type, population (i.e., site in Western Australia from which *Emex australis* seeds used in the screen house experiment were sourced), scarification, and light availability.^a

| Experiment | Factor | | Germination | P | LSD |
|-------------------------------|---------------|--------------|-------------|--------|-------|
| 2018 experiments ^b | Seed type | Aerial | 37.1 | 0.442 | 13.59 |
| | | Subterranean | 41.7 | | |
| | Location | Field | 18.6 | <0.001 | 13.59 |
| | | Screen house | 60.2 | | |
| 2020 experiment | Seed type | Aerial | 19.6 | 0.076 | 4.04 |
| | | Subterranean | 23.2 | | |
| | Scarification | Yes | 23.2 | <0.001 | 2.03 |
| | | No | 17.4 | | |
| | Light | Light | 24.5 | 0.003 | 4.04 |
| | | Dark | 18.3 | | |
| | Population | Grass Valley | 19.2 | 0.036 | 3.2 |
| | | Wongan Hills | 16.0 | | |
| | | York | 23.8 | | |
| | | Moora | 22.9 | | |
| | | Bolgart | 25.2 | | |

^aP and LSD values are included for separation of means within each factor. There were no significant interactions between factors, except for the interaction between seed type and light in the 2020 experiment, displayed in Figure 5.

^bThe 2018 experiments only used the Wongan Hills population.

conditions, whereas aerial seeds had similar germination to subterranean seeds in light conditions and reduced germination in the dark. Javaid and Tanveer (2014) found a light requirement for

germination in *E. australis* seeds but did not differentiate between aerial and subterranean seeds and were probably only using aerial seeds. The current research indicates that the subterranean seeds had

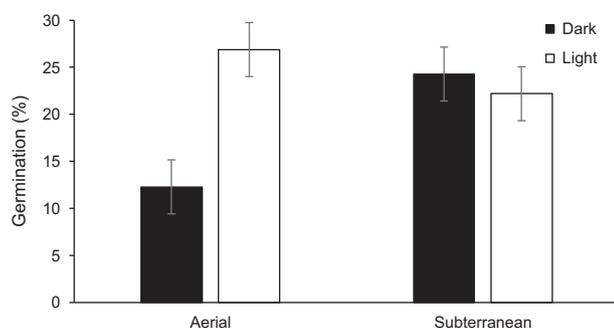


Figure 5. Germination (as a percent per petri dish) of aerial or subterranean seeds in dark conditions (petri dishes wrapped in foil) or light (petri dishes exposed to a 12-h light/dark photoperiod). Vertical bars indicate the standard error of the difference of the means ($P < 0.001$, LSD: 5.71).

similar emergence in dark and light conditions. Scarification increased germination of both seed types (Table 4). The current research has determined that subterranean seeds have similar viability and germinability to those of aerial seeds and are not dependent on exposure to light. However, scarification increases germination of both seeds, and the subterranean seeds at depth are probably less likely to be physically damaged than aerial seeds on the soil surface. It is possible that if seeds were stored for longer to achieve greater dormancy release, the impact of light and scarification would become less apparent. While significant, the differences between these factors are smaller than the differences in emergence in the field resulting from burial or longer-term dormancy release in previous studies (Cheam 1996; Panetta and Randall 1993). Further research is required to determine how these factors interact to impact timing of emergence of subterranean and aerial seeds in arable fields and the impact of each seed type on the population ecology of *E. australis*.

Germination of seed from the 2018 experiments was affected by location, with seeds from plants grown in the 2018 screen house experiment having higher germination than seeds from plants grown in the 2018 field experiment. However, germination of field seed in 2018 (18.6%) was similar to germination of some populations from the screen house experiment in 2020. In 2020, germination was slightly different among the five populations grown in the screen house, ranging from 16% to 25.2% (Table 4). However, the variation in germination in the current study is comparable to results in earlier studies in which initial germination following afterripening ranged from 11% to 95%, although field emergence is much lower than germination in controlled conditions and dependent on temperature and burial (Cheam 1996; Panetta and Randall 1993). The seed from different populations used in the 2020 screen house experiment were from parental plants that grew in different locations. Therefore, the seeds were exposed to different environmental conditions at development, which may affect dormancy (Finch-Savage and Leubner-Metzger 2006). For example, high temperatures or water stress during seed development have been linked to reduced dormancy (increased seed germination) in other species (Benech-Arnold et al. 1992; Fenner 1991; Finch-Savage and Leubner-Metzger 2006). Prior research has established that *E. australis* seeds have high initial dormancy and cycle in and out of dormancy following afterripening (Panetta and Randall 1993). As a winter annual in a Mediterranean climate, *E. australis* obviously needs to produce seeds that remain dormant over the summer fallow (Cheam 1996; Panetta and Randall 1993). However, subterranean seeds have reduced opportunity to disperse

away from the parent compared with aerial seeds (Gilbey et al. 1998; Lemerle 1996; Michael et al. 2010). Therefore, subterranean seeds would also need to avoid germinating while the parent plant survives to avoid excessive competition with the mature plant.

Preventing development of subterranean seeds is likely to be important for growers or managers aiming to eradicate *E. australis*. However, the main goal of an integrated weed management program should be prevention of aerial seed production. If *E. australis* plants are not controlled, they may produce up to 1,100 aerial seeds; enough to ensure that 13 to 30 subterranean seeds per plant will not make a significant difference to the soil seedbank in the following year. Further, one of the main reasons for controlling this species is to prevent crop yield reduction from weed competition, which would not occur from *E. australis* plants at the 4-leaf stage. A second reason for control is to prevent economic loss caused by the mature, dispersing seeds, that is, financial loss from damage caused to livestock when they step on the seeds or contamination of hay and grain at harvest (CABI 2020; Michael et al. 2010). The subterranean seeds are much less likely to damage livestock or contaminate grain. However, even if subterranean seeds are returned to the soil surface by the crop-sowing operation, they are less likely to damage livestock due to their bilateral symmetry rather than the radial symmetry of the aerial seeds, which ensures that one spine of the aerial seed is always pointing up (Gilbey et al. 1998; Figure 1). Subterranean seeds also have shorter spines than aerial seeds (Gilbey et al. 1998). Therefore, if growers control young *E. australis* plants after subterranean seed set but before aerial seed set, they will not eradicate the weed, but will avoid most economic loss resulting from this species.

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