Methods to select areas to survey for biological control agents: An example based on growth in relation to temperature and distribution of the weed *Conyza bonariensis*

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Short title: Selecting areas to survey for biological control agents

ABSTRACT

A novel approach for selecting areas to survey for biological control agents, incorporating climate and a hypothesised biological control agent, is demonstrated using the target weed *Conyza bonariensis* (Asteraceae). This weed has become important in Australian cropping regions due to its persistence and herbicide resistance, and it is also increasingly an environmental weed. Both are reasons for the investigation of biological control options. We developed a species niche model for *C. bonariensis* in CLIMEX based on parameters informed by plant growth and distribution of the species in the Americas. A hypothetical biological control agent (HBCA-cold) was proposed that has its ideal growth range 5 °C below that of the weed, so as to favour development of the agent over that of the weed in parts of Australia. The southern part of the weed's native distribution in Argentina, Chile and the highlands of Ecuador and Columbia were identified as the most suitable areas for surveys that take into account both the climate suitable for the HBCA-cold and the target regions in Australia. This was compared to a model (HBCA-hot) that had an ideal growth range 5 °C above that of the weed, but which identified potential areas for surveys in South America that were not climatically aligned with the main regions of the weed's economic impact in Australia. This species distribution modelling method allows for prioritisation of search areas for biological control agents in the case of widespread target species such as *C. bonariensis.*

Keywords: Biological control, Cropping weed, Mechanistic model, South America, Australia

1. Introduction

Biological control of weeds has had a long history of matching climates between the known weed-infested area and a source area in the native range that is then surveyed for potential biological control agents (Fisher et al., 2011; Robertson et al., 2008; Sutherst and Maywald, 1985). Climates can be matched efficiently and quickly using methods such as Klimadiagramm Weltatlas (Walter and Leith, 1960-67), the Köppen–Geiger climate classification scheme (Kriticos et al., 2012) or a range of correlative modelling techniques that are available. However, the matching of climates method has been recognised as failing to account for weed invasion into novel climates, both under current and future projected climates that are relative to the native range (van Klinken et al., 2009; Webber et al., 2011). In addition, the matching of climates method assumes that the weed species has reached its invasion potential in the area of introduction, which is often not the case. Despite these limitations, a decision inevitably needs to be made on where to survey for biological control agents and that decision is often, at least partly, made on the basis of identifying climates similar to the introduced region of the weed.

Biological control is being considered in Australia for controlling the cropping and environmental weed *Conyza bonariensis* (L.) Cronquist (Asteraceae), an annual herb native to South America. The lack of close relatives of the weed in the Australian native flora increases the likelihood of finding agents that will be suitably host-specific. In addition, potentially host-specific pathogens and plant-feeding insects are known to be associated with the genus *Conyza* in South America (e.g. the rust, *Aecidium conyzae-colombiensis* Pardo-Cardona (Pardo-Cardona, 2000) and the tephritid, *Trupanea bonariensis* (Brèthes) (McKay and Gandolfo, 2001)), however, no systematic surveys have been carried out on *C. bonariensis* for suitable agents.

66 *Conyza bonariensis* has a widespread native distribution and comprehensive
67 surveys for biological control agents over such a large area would not be feasible due
68 to the time and resources required. Therefore, a prioritisation scheme needs to be
69 established for the location of initial surveys. At a practical level, there are often
70 inexplicable gaps in the recorded distribution of a species in the region of origin
71 without an ecologically plausible reason. These notable absences could be due to lack
72 of surveys or a lack of access to the full set of species distribution records, although
73 these areas should still be considered in any potential surveys.
74 Species niche modelling methods, such as CLIMEX, that compare locations
75 are appropriate for assessing these issues. The "Compare Locations" option in
76 CLIMEX models the species climate suitability based on calculating the weekly
77 growth of a species by the physiological response to temperature and moisture and
78 integrating this annually (Sutherst and Maywald, 1985). The potential for growth is
79 then assessed for a region using climatic data. The CLIMEX method has already been
80 used extensively in biological control (Scott 1992; Julien et al., 1995; Dhileepan et al.
81 2006) and has the advantage of consideration of the organism's biology, in contrast to
82 climate matching approaches only.
83 In this paper we developed a distribution model for *C. bonariensis* using the
84 mechanistic modelling method CLIMEX. We developed the parameters for CLIMEX
85 from the plant's response to temperature based on experimental assessments of seed
86 germination and plant growth. We also estimated the soil moisture and environmental
87 stress values based on the observed distribution and knowledge of the biology of the
88 species. The model produces an Ecoclimatic Index (EI), which is a measure of how
89 favourable a location is for plant growth. In this paper we explore a novel approach to
90 help survey for biological control agents. Firstly, we map the potential distribution of

the weed in its native and introduced range. This can then be used to define the overall area for the survey of potential biological control agents. Secondly, we establish a novel approach of modelling the potential distribution of a hypothetical biological control agent (HBCA) that responds to a different temperature regime to that of the target weed. The latter can then be used to define a subset of the area to firstly survey in the native habitat and secondly to release in the weed-infested region.

2. Materials and Methods

2.1 Target weed species

Conyza bonariensis, a weed of disturbed areas and wasteland, is the most widespread *Conyza* species in Australia (Burry and Kloot, 1982). The abundance and impact of *C. bonariensis* is increasing in minimum tillage farming systems in southern and eastern Australia. This change is thought to be a result of better germination conditions, and therefore it is one of the most difficult weeds to control in these systems (Wu et al., 2007). The weed can produce large numbers of viable non-dormant seeds that germinate all year round with the potential to complete multiple lifecycles in a year (Wu et al., 2007).

Conyza bonariensis has evolved resistance to a range of herbicides across four different modes of action in eleven countries including Australia (Heap, 2015). Control in summer becomes increasingly difficult as the season progresses as weeds are often under severe moisture stress at the time of herbicide application, therefore reducing herbicide efficacy (Wu et al., 2007). Tillage is effective in suppressing plants although mowing is not, as it encourages lateral branching from the base of the plants,

115 hardening them off (Wu et al., 2007). These factors point to the need to consider
116 biological control in cropping situations.
117 In addition, *C. bonariensis* is an increasing problem as an invasive plant in
118 conservation areas such as urban bushland in Sydney (Clements, 1983). In native
119 ecosystems there are very limited options for control using herbicides or hand
120 weeding, which makes biological control the method of choice.
121
122 *2.2 Seed germination and plant growth in relation to temperature*
123 Germination experiments were performed on *C. bonariensis* seeds to provide an
124 estimate of the temperature parameters to use in CLIMEX. Seed heads from mature
125 plants were harvested during January 2009 from remnant vegetation and a sports field
126 at Merredin, Western Australia (31°28'27.33"S, 118°16'52.08"E). Seeds were stored
127 in paper envelopes under laboratory conditions until required for germination or
128 growth experiments.
129 The effect of temperature upon the germination of seeds was assessed in 90
130 mm diam. Petri dishes by placing ten seeds on two layers of filter paper (Whatman
131 #1) moistened with regular applications of tap water. Each Petri dish was individually
132 placed within a clear zip-lock polypropylene bag to reduce evaporation. Five Petri
133 dishes per temperature were placed in Lindner and May (Windsor, Queensland)
134 environmental chambers (Model LMRIL-5) run at 4.0, 11.9, 14.4, 18.7, 24.5, 27.8,
135 35.2 and 40.3 °C (the temperature was verified by data loggers in each chamber, S.E.
136 of hourly means ranged from 0.01 - 0.04 °C) and exposed to a 14 hr daily
137 photoperiod. The lighting in the chambers was provided by 3 x 30 w fluorescent
138 globes (5000 k colour) located on the internal back wall of the units.
139 Photosynthetically active radiation levels (PAR) measured with a Decagon Accupar

LP-80 fitted with an external sensor, varied within the chambers depending upon the distance of the plants to the globes and the age of the globes, but ranged from 24 to 170 microMol/m²s. Trays holding the petri dishes were rotated each inspection so that all seeds should have received approximately the same light regime during the experiment. A further five Petri dishes were placed on a bench in a glasshouse as a control (glasshouses had 70% shade cloth fitted with PAR of up to 400 microMol/m²s possible). Germination, defined as when the radicle penetrated the seed coat wall, was assessed daily. The trial was terminated after either all seeds within a Petri dish had germinated or at 17 days, as at this time fungal growth was appearing on seeds in the warmer chambers. Only seeds that were observed to be imbibed after the first day were included in the analysis. All non-germinating seeds were examined to see if the endosperm was intact and firm or if they had collapsed and rotted sometime prior to or during the experiment (possibly non-viable seeds).

After analysing the first set of data, we considered the period of time that the seeds had been held at 4.0 °C was too short for assessing germination potential. As fungal growth was not a problem at this temperature, a second batch of five Petri-dishes was setup at 4.0 °C and allowed to run for 117 days. These were assessed every two or three days.

2.3 Plant growth in relation to temperature

On 29th October 2009, seeds were planted into 48 cell seedling trays (Rite Gro Kwik Pot) containing approximately 50 ml per cell of a modified University of California potting mix (coco peat was substituted for sphagnum). Two seeds were placed on the soil surface of each cell and lightly covered with additional potting mix. Initial emergence was recorded on 1st November 2009 and by 4th November 2009 the

majority of cells contained two emerged seedlings. Seedlings were then randomly thinned to one seedling per cell. Complete trays (i.e. 48 seedlings) were placed into Lindner and May environmental chambers run at 6.8, 9.5, 15.7, 18.5, 24.4, 28.0, 35.1 and 38.6 °C on 14th November 2009 (the temperature was verified by data loggers in each chamber, S.E. of hourly means ranged from 0.01 - 0.16 °C). A further 48 seedlings were also placed into a glasshouse at this time in order to determine growth under normal light conditions.

The seedling trays where placed upon a solid tray and the plants checked every two to three days for any mortality of individuals, and to check if they needed to be rewatered (bottom watered). They were top watered with liquid fertiliser (Ducolos Soluble Fertiliser Soluplant Starter NPK + TE (15:13.1:12.5+ME) at a rate of 1 Tbsp/4l) initially when the experiment commenced and then subsequently at monthly intervals.

Plant size (based on leaf area and on total stem lengths) was estimated at the beginning of the experiment and at approximately monthly intervals. The number of leaves, average length and width (mm) of live leaves was measured using a calliper or ruler. Average leaf area was calculated by average leaf width x average leaf length x 0.65, the latter value a correction factor based on the shape of the leaves. Daily growth rates for the plant foliage were based upon change in total leaf area (number of leaves x average leaf area) over the maximum number of months that the plant was alive. The growth rates are expressed as per day as different individuals had different longevities. The total stem length of the plant was measured as the sum of the length of the stems together with any branches on these stems. Daily growth rates for the plants stem were again based upon changes in stem lengths over the maximum period that was possible for each plant. Plants that died before the first monthly

measurements were given a value of 0% growth.The experiment was terminated early March 2010, when several plants in the glasshouse had set seed (approximately three months after the experiment started). Over a one week period, plants were measured and washed to remove any soil, then oven dried at 65 °C in paper bags to calculate dry weights. Growth rates based upon dry weights were expressed per day rather than per experimental period to allow for these variations in time, however plants that died before the end of the experiment (= before maturity) were given a 0% growth rate (for biomass) so as to prevent any bias to the data from selective mortality within the chamber as only the bigger individuals within a cohort tended to survive the extreme temperatures whereas all individuals survived any favourable temperatures.

2.4 Mapping of distribution

The distribution data for *C. bonariensis* were depicted in two ways. Firstly, if the exact location of the species was known (i.e. specific co-ordinates), then it was indicated on the map as a dot (see Fig. 1). Several online databases were used to determine the worldwide distribution of point sources for *C. bonariensis* including Australian Virtual Herbarium (www.chah.gov.au/avh), Global Biodiversity Information Facility (www.data.gbif.org), Specieslink (http://splink.cria.org.br), TROPICOS (www.tropicos.org) and The World Biodiversity Information Network (www.conabio.gob.mx/remib). We included the synonyms as listed in Wu et al. (2007) when investigating records of the species distribution. We cleaned the data by removing 4399 records out of 9719 that were either errors, duplicates or had inadequate information to indicate a specific location.

Secondly, if the distribution was known only on a regional basis, then the region was indicated on the map according to Brummitt's "World Geographical

Scheme for Recording Plant Distributions, Plant Taxonomic Database Standards No. 2" (Level 4, basic recording units) (Brummitt et al., 2001). The Brummitt regions map was based on distribution information from the Euro+Med PlantBase (www.emplantbase.org), TROPICOS (www.tropicos.org), United States Department of Agriculture Plants Database (www.plants.usda.gov), Rios and Garcia (1998) and Šída (2003). Regions were categorised as either being in the native distribution or the introduced distribution. Brummitt regions with the presence of a point source record, but no literature source were also categorised as native or introduced depending on the status of neighbouring regions. We note that *C. bonariensis* is a very widespread weed and these two methods, despite being drawn from major literature sources, are likely to be an under-estimate of the true global distribution.

2.5 The CLIMEX model

CLIMEX contains a parameter set of five meteorological variables: average minimum monthly temperature (Tmin), average maximum monthly temperature (Tmax), average monthly precipitation (Ptotal) and relative humidity at 09:00 h (H09:00) and 15:00 h (H15:00). These are used in the "Compare Locations" option to define weekly and annual indices that determine the species response to temperature and soil moisture. CLIMEX calculates an annual Growth Index (GI_A) based on the growth of a species under favourable conditions of temperature, moisture and light. Stress indices (cold, hot, wet and dry) and their interactions may also be added to the model to indicate species restriction during unfavourable conditions. The Growth and Stress indices are combined to create the Ecoclimatic Index (EI), a measure of the favourableness of a particular location for the species.

239 Details of the methodology and parameters used in CLIMEX and comparisons
240 with correlative methods are discussed in Webber et al. (2011) and Yonow and
241 Sutherst (1998). Climate (recent historical data centred on 1975) was modelled using
242 the CliMond 10' gridded world climate dataset, as described in Kriticos et al. (2012).
243 Further background to these methods can be found in Kriticos et al. (2012), Webber et
244 al. (2011) and Michael et al. (2012).
245
246 *2.6 Hypothetical biological control agent*
247 We assumed that any host-specific herbivores or pathogens would have a distribution
248 within the climatic range of the host, *C. bonariensis*. To develop a model for an ideal
249 HBCA, we started with the same model parameters for *C. bonariensis* and chose, as
250 an example, that peak growth (i.e. values of DV1 and DV2, Table 1) of the agent
251 occurred at a temperature 5 °C lower than the temperature for peak growth of the
252 weed (HBCA-cold). Our experience with some biological control agents of weeds of
253 Mediterranean-type climate (e.g. Scott and Yeoh, 1999) indicated that a delayed
254 growth response to temperature over winter in southern Australian conditions results
255 in the weed out-growing any damage from the agent (optimal temperatures for agent
256 growth > optimal temperatures for weed growth). Ideally the agent should develop
257 faster than the weed (cf. Myers, 1980), in our case, during winter. The latter situation
258 potentially provides control of the weed (agent optimal growth temperature < weed
259 optimal growth temperature). In addition, we chose 5 °C so that a difference between
260 weed and agent would be clearly demonstrated. By way of comparison we also
261 modelled the opposite scenario, that of a HBCA that responds favourably to a
262 temperature 5 °C greater than that preferred by the host plant (HBCA-hot).
263

2.7 GIS methods and statistical techniques

We used ESRI ArcView Version 10.2 to generate the maps for this study. A global fishnet provided with the CliMond dataset (Kriticos et al., 2012) at a grid size of 10' was used to visualise the CLIMEX output. A chi-squared test was used to test the model projection for statistical significance as described in Webber et al. (2011). Calculations of modelled sensitivity and prevalence also follow Webber et al. (2011).

3. Results

3.1 Seed germination in relation to temperature

Seeds were able to germinate within the range of 4.0 to 27.8 ℃ (Fig. 2A). Total germination was slightly lower at 4.0 ℃ than at higher temperatures, averaging 64% within 117 days (Fig. 3). Germination was also considerably delayed at 4.0 ℃ in comparison to higher temperatures (Fig. 3). For seeds within the growth chambers at 11.9 to 27.8 ℃, total germination was consistently high, averaging 89% per petri dish (range 60-100%). In the glasshouse, where temperatures averaged 19.6 ℃ (range 9.9 to 34.0 ℃) and there was natural light, germination was 82%. At 35.2 ℃ or above seeds imbibed, but failed to germinate and consequently died. The germination tests indicate a lower temperature threshold of 3.0 ℃ (Fig. 2A). The optimal range of temperatures for germination was estimated for the CLIMEX model to be 11.9 to 24.5 ℃ and seed at or above 35 ℃ died, giving this constant temperature as an upper limit for germination.

3.2 Plant growth in relation to temperature

The biomass production of plants growing within the chambers was estimated to be optimal for plants in the 13 to 20 ℃ temperature range (Fig. 2B). However, plants

289 grew better in the glasshouse than in the environmental chambers with approximately
290 5 times as much biomass being accumulated during the experimental period (11.7 ±
291 0.68 mg/plant (n=48) dry weight in glasshouse vs 2.8 ± 0.36 mg/plant (n=48) at 15.8
292 °C ; the temperature chamber with the highest biomass accumulation rates).

293 Long-term exposure (months) to 30 °C or medium term exposure (weeks) to
294 >37 °C was detrimental to the health of plants (Fig. 2C). No plants survived to the end
295 of the trial at 37 °C and only 1 seedling (out of 48) survived at 28 °C, although plants
296 were, able to persist in the short term (1 to 2 months). At 24.4 °C, 83% of the plants
297 were still alive after 21 days and 50% after 111 days. Some mortality also occurred at
298 lower temperatures (6.9 °C) but individuals were able to survive for more than 87
299 days at this temperature, indicating tolerance of cold temperatures (Fig. 2C).

300 Growth of seedlings did not occur at 6.9 °C, so the lower developmental
301 threshold temperature (DV0) was initially estimated to be approximately 7.5 °C. The
302 upper developmental threshold temperature (DV3) was approximately 33 °C for
303 vegetative growth and 20 °C for reproductive growth. Based upon some leaf
304 production occurring at all chambers from 9.5 °C to 37.2 °C and a relative even but
305 higher rate of leaf production occurring between 15.7 °C (0.15 ±0.050 (SE)
306 mm^2/day/plant, n=48) and 24.4 °C (0.12 ± 0.025 (SE) mm^2/day/plant, n=48), optimal
307 vegetative growth was estimated to occurred between 13 °C and 27 °C. Although
308 stems or flowers potentially may have been produced if the plants were allowed to
309 grow longer, stem production for plants during the experimental period differed to
310 leaf production by being observed in a far more restricted temperature range
311 compared to what was considered optimal for leaf production. There were no stems
312 produced at or below 9.5 °C. The average rate of stem production at 15.7 °C (0.009
313 mm/experimental day) was approximately four times that observed for plants within

314 the chamber running at 18.5 °C and no stem production occurring on plants within the
315 chambers set at 24.4 °C or hotter.
316 We continued the experiment until the stage where some of the plants in the
317 glasshouse had produced open flowers (4 months after emergence). Many of the other
318 plants in the glasshouse treatment also had flower buds (14 out of 44 plants).
319 Although some of the plants in the temperature chambers had produced stems, none
320 had buds. Based upon 4 of the 48 individuals that produced flowers in the glasshouse,
321 the average minimum degree-days above a lower developmental threshold (DV0)
322 value of 7.5 °C was 2422 °D from emergence to flowering, information that could be
323 used to inform the CLIMEX model. The average time until flowering was 105 ± 3.3
324 days after the start of the experiment (and they were on average 3 days old at the start
325 of the experiment).
326
327 *3.3 Native and introduced distribution*
328 *Conyza bonariensis* is generally considered a native of South America with its status
329 in Central America sometimes listed as native or as introduced. We have followed
330 Rios and Garcia (1998) and Strother (2006) in indicating that it is introduced to
331 Mexico and USA respectively. It is widespread throughout the coastal regions of
332 southern USA, although it is not present in the cooler regions of Canada (Weaver,
333 2001) and central USA (Strother, 2006) (Fig. 1). Likewise, the plant is absent in most
334 of Scandinavia, being present as an ephemeral species in southern regions (Gederaas,
335 et al. 2012; Karlsson, 1998). Elsewhere, the plant is widespread in Europe, the
336 Mediterranean region, southern and eastern Africa and present in Asia. Within
337 Australia *C. bonariensis* is found throughout most of the continent, is sparse in central
338 regions and tends to be mainly distributed in more temperate regions (Fig. 1).

339

340 *3.4 Development of CLIMEX Compare Locations model*

341 Initial temperature and degree-day parameters were determined by germination,
342 glasshouse and laboratory studies in conjunction with published data (Wu et al., 2007)
343 (Table 1). The model was trained using the iterative process of CLIMEX guided both
344 by the native distribution in South America and the possible range extension to North
345 America (Fig. 1). Estimates of the lower temperature threshold (DV0) derived from
346 seed germination, plant growth and published studies ranged from 3 to 7 °C (Fig. 2),
347 but a single value is needed for the CLIMEX model (Table 1). A lower development
348 threshold temperature of 4 °C was chosen because if falls within the range of our
349 results (Fig. 2) and those of Wu et al. (2007), and also because it enabled the
350 Temperature Index in CLIMEX to map records at the extremes of the native and
351 range extension distribution (both North and South America). Zambrano-Navea et al.
352 (2013) derived a base temperature of 10.6 °C for *C. bonariensis*, but this was
353 calculated from germination experiments that were run at only three temperatures, 15
354 °C or higher. Modelling using 10.6 °C at DV0 excluded a major part of the observed
355 distribution, consequently this temperature value was not used in the modelling
356 process. The upper temperature threshold (DV3 = 33 °C) was based on the upper
357 temperature above which growth did not occur and the ideal range 15-25 °C was
358 based on the temperatures where increased growth occurred (Fig. 2).

359 The lower moisture index parameters (SM0 = 0.2) were set to include Mexico
360 and drier parts of Central America whilst the upper soil moisture threshold value
361 (SM3 = 1.6) was set to include collection records on the east of the Andes Mountains
362 in Ecuador. Zambrano-Navea et al. (2013) calculated a hydrothermal model for *C.*
363 *bonariensis* with germination ceasing at a constant water potential (Ψ) of -1.06 MPa

(MegaPascals) and 50% germination occurring at -0.7 MPa. While the CLIMEX manuals do not define an explicit relationship between water potential and the Moisture Index, these values also indicate a SM0 of around 0.2. Using this value in the model excludes the distribution of *C. bonariensis* in central Australia (it is included using SM0 = 0.1). However, the presence of *C. bonariensis* in dry regions is mostly related to microhabitats where moisture accumulates (roadsides, gardens). In support of this observation, the addition of 1 mm of daily irrigation in winter (in addition to SM0 = 0.2) is sufficient for the model to include the central Australian distribution.

The model based on these temperature and moisture values included most of the records on the American continents, but not all. Initially, the value for degree-days was set at 2768 as indicated from the plant growth experiments (and assuming a lower development threshold temperature of 4 °C). However, regions with collection records on the west coast of USA and southern South America (Fig. 4) were excluded due to the degree-day value. Therefore the degree-days were progressively reduced to 1900 so that these points were included in the CLIMEX model. Reducing the limiting low temperature threshold (DV0) did not have the same result on the model unless unreasonable values near to 0 °C were used.

The model was further refined by two stress parameters. Cold Stress was used to define the northern-most limits to the distribution in central North America (Fig. 4). Hot Wet Stress was used to improve the projection of the distribution in tropical regions of South America (Fig. 4).

The final parameterised CLIMEX model of the distribution of *C. bonariensis* covers the known native and introduced distribution in the Americas (Fig. 4). The CLIMEX model (Table 1) had high sensitivity (the proportion of all test locations

correctly modelled as occurring in climatically suitable areas) of 93% in the native (South America) region, and high prevalence (the proportion of the model universe estimated to be climatically suitable) of 0.70. That is, *C. bonariensis* is widespread in South America. The model also shows the absences in North America (we cannot be certain about the true absences in Central and South America) and that considerable range expansion beyond the observed distribution is possible in central and eastern parts of North America (Fig. 4). Modelled prevalence for the Americas was 0.32 and the sensitivity 93%. The model projection was statistically significant ($P<0.0001$) when tested against known distribution records in the Americas.

The CLIMEX model could encompass more of the data points in northern Europe by decreasing the degree-day value. Otherwise the model suitability includes the vast majority (99%) of known records from Africa, Asia and Europe (Fig. 4). The model suitability also covers the Australian distribution records (aside from those in drier regions) (Fig. 5) showing a high level of sensitivity (0.91). The model projection was statistically significant ($P<0.0001$) when tested against known distribution records in Australia. The modelled prevalence was 0.28 for Australia.

3.5 Hypothetical biological control agent

We made the CLIMEX parameters for *C. bonariensis* and the hypothetical biological control agent (HBCA) identical except for the ideal range of temperatures (DV1 and DV2) in the HBCA (Table 1) which were either 5 °C colder or hotter. This means that the projected suitable area of the HBCA (EI > 0) is identical to that of the weed. Differences between the two are shown by an increase or decrease in the EI within the area suitable for the weed (Fig. 6 and 7). The area most suitable for the weed (EI > 30) is indicated by shading on the maps (Fig. 6 and 7).

The projection to South America of relatively high levels of EI for the HBCA - cold, contrasted to similar EI values for the weed, identifies regions in the east of Argentina, central Chile and along the eastern foothills of Andes Mountains that are highly suitable for the HBCA (Fig. 6), thus indicating suitable regions for prospecting. In Australia, potentially suitable release areas for HBCA – cold were found in Western and eastern Australia (Fig. 6). These overlap with major grains producing regions.

For comparison, Fig. 7 shows the results of a CLIMEX model for HBCA - hot that responds to a 5 °C warmer temperature. In the native region, South America, that is suitable or optimal for *C. bonariensis* (Fig. 5), the potential areas of exploration are indicated for southern Brazil, Uruguay, and northern Argentina. However, a HBCA – hot is mis-matched to most of the cropping region of Australia, except in southern Queensland.

4. Discussion

We demonstrate a new approach to the long established method of selecting climatically matched areas for the search for biological control agents, identifying regions highly suitable for growth of a HBCA. This method is applicable to any target for biological control, weed or arthropod. The parameter set in Table 1 enables the reproduction of the results and their modification when new information becomes available (e.g. development rate in relation to temperature for a specific biological control agent). However, we recognise there are a number of caveats. Firstly, the models assume that the weed distributions are determined by climate. While ultimately this must be true, the models do not take into account biotic and other

439 abiotic factors such as soil associations (Michael et al., 2012). The other assumption is
440 that it is preferable to search for biological control agents in the region most
441 favourable for weed growth as opposed to the edges of the weed distribution.
442 It can be argued that biological control searches should look at the edges of
443 species realised distributions or where the weed is sparse, because that is where
444 distribution- or abundance-limiting agents might be found. We are aware of one study
445 that examined the distribution edges only and that was because of political barriers
446 restricting studies of *Euphorbia* species to the western edge of their European
447 distribution (Gassmann and Schroeder, 1995). Studying the distribution edges is a
448 valid approach, but it requires a sound knowledge of the species distribution model
449 based on climate. Only then is it possible to take into account other factors (i.e.
450 edaphic or abiotic) so that it is reasonable to focus on potential biological control
451 limits to the distribution and abundance. Indeed it would be possible to use a
452 CLIMEX Compare Locations model to identify two such regions. Firstly, differences
453 between the fundamental niche approximated by the CLIMEX model and the realised
454 niche may indicate the presence of biotic range-limiting factors. Secondly, if the
455 distribution and abundance are well documented, then low abundance in areas with
456 high EI values may indicate an abundance limiting factor. Central Argentina would be
457 such a region for either reason (sparse records or edge of observed distribution, high
458 EI). It is thus possible to test these ideas and the HBCA model by structured surveys
459 in the various regions.
460
461 *4.1 Development in relation to temperature*
462 *Conyza bonariensis* seed in our study germinate at any temperature above a lower
463 threshold of about 3 °C. The ability to germinate at low temperatures is not shown in

earlier studies of *C. bonariensis* (Wu et al., 2007; Karlsson and Milberg, 2007; Zambrano-Navea et al., 2013) because the length of germination trials (between 10-17 days and up to 48 days) was not long enough to detect germination at these low temperatures due to insufficient degree-days to complete this stage of development. This also implies that there is no physiological dormancy related to temperature; a conclusion arrived at by the other studies. However, high temperatures (given the high humidity in Petri-dishes) were fatal to seeds.

The measurement of growth in relation to temperature indicates that this weed has a high tolerance of cold temperatures. Germination occurs at any time of the year, provided it is warm enough. Subsequently rosettes tolerate winter conditions typical of Mediterranean-type climates such as found in southern Australia. Likewise Zambrano-Navea et al. (2013) in southern Spain and Shrestha et al. (2008) in California report that germination could occur at any time of the year. In both areas plants bolt and produce flowers and seed from when warmer conditions return through to late autumn (Shrestha et al., 2008; Zambrano-Navea et al., 2013). This supports the idea of searching for a biological control agent that is damaging to the plant in winter before bolting and seed set.

Our experimental work indicated a relatively high degree-day value when compared to annual herbs. The degree-day value is likely to be an over-estimate because of delays caused by meeting the day length requirement for bolting. Plants germinating in spring or early summer, given the rapid accumulation of degree-days under warm conditions, could flower and produce seed, with considerably less degree-days than plants that germinate in autumn. In more subtropical areas growth would be mainly during winter as also observed by Wu et al. (2007) and higher degree-days may be required. Further experimental work is required both on the effect of day

length and on germination time to improve our understanding of the amount of degree-days required for plant development.

4.2 Distribution in Australia

Regions where *C. bonariensis* is an emerging weed, such as southern Western Australia (Owen et al., 2009), are clearly highly favourable for the weed. The widespread distribution in Australia across a range of climate types (including tropical, arid and temperate regions) also makes it questionable to do a simple matching of climates, such as would have been done in the past. Here we show how plant growth parameter can be used to derive a species distribution model that covers a range of climate types in Australia where the seed is found.

4.3 Selection of prospection area for biological control

Prospecting in South America took years before suitable, highly-successful agents were found for early biological control programs (e.g. *Salvina*, *Opuntia*) (Dodd, 1940; Julien, 2012). *Conyza bonariensis* is a very widespread species and this is reflected in the prevalence value of 0.70 in South America, indicating that most of the continent (prevalence ranges from 0 to 1.00) falls within a suitable climate for this species. Clearly, it is not feasible to prospect all this area for biological control agents. In the current funding environment, efficiencies would be sought, such as selecting priority areas for prospection.

This is the first time a HBCA has been proposed at the start of a biological control program. For this study only one attribute of the agent was chosen, that of a more favourable development at lower temperature. Other options could be

investigated such as a more favourable response to lower soil moisture or cold tolerance.

Three areas were identified as optimal for prospecting, the Andes, Chile and Argentina. A strategy could be to give priority for investigation to Argentina or Chile, before the much smaller regions of the Andes in Peru and Columbia. Another advantage of using a CLIMEX model is that growth (GI) is estimated weekly throughout the year. This means that the model can also be used to identify suitable periods in the year for prospecting.

A distribution model for *C. bonariensis* will also form the basis for future models for biological control agents. The models could be used to identify regions for release of biological control agents in Australia (i.e. to refine the models shown in Fig. 6 and 7). *Conyza bonariensis* is most problematic in grains producing areas in southern Queensland and northern NSW. In these regions the HBCA – cold model is either similar or better than the weed (Fig. 6). To help this process it would be useful to include in future studies of potential agents, further aspects of physiology that build towards informing CLIMEX model parameterisation (Scott and Yeoh, 1999).

Given the potential problems with herbicide resistance and the increasing importance of *C. bonariensis* in agriculture and the environment, there is need for research on alternative control methods such as biological control. In addition, biological control is the only long term solution to address the increasing importance of *C. bonariensis* as a weed of conservation areas. The development of a species bioclimatic model in CLIMEX enables the identification of areas where the weed could be found. A hypothetical biological control agent, developed from the *C. bonariensis* model, enables the identification of specific areas in South America most

suited for a search for biological control agents with attributes that may be important for achieving control of the weed.

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637

638 Table 1. CLIMEX parameters values used for modelling the distribution of *Conyza*
639 *bonariensis* based on the temperature requirements for development and native
640 distribution. The model parameters for the hypothetical biological control agent differ
641 to *C. bonariensis* by a five degrees lower or higher optimal temperature.

Index	Parameter	Values			Units[a]
		Conyza bonariensis	Hypothetical biocontrol agent (cold)	Hypothetical biocontrol agent (hot)	
Temperature	DV0 = lower threshold	4	4	4	°C
	DV1 = lower optimum temperature	15	10	20	°C
	DV2 = upper optimum temperature	25	20	30	°C
	DV3 = upper threshold	33	33	33	°C
Moisture	SM0 = lower soil moisture threshold	0.2	0.2	0.2	
	SM1 = lower optimum soil moisture	0.4	0.4	0.4	
	SM2 = upper optimum soil moisture	1	1	1	
	SM3 = upper soil moisture threshold	1.6	1.6	1.6	
Cold stress	TTCS = temperature threshold	-3.5	-3.5	-3.5	°C

	THCS = cold stress accumulation rate	-0.001	-0.001	-0.001	$Week^{-1}$
Hot wet stress	TTHW = temperature threshold	29	29	29	°C
	MTHW = moisture threshold	1.0	1.0	1.0	
	PHW = hot wet stress accumulation rate	0.01	0.01	0.01	$Week^{-1}$
PDD	Number of degree-days above DV0 necessary to complete one generation	1900	1900	1900	°C days

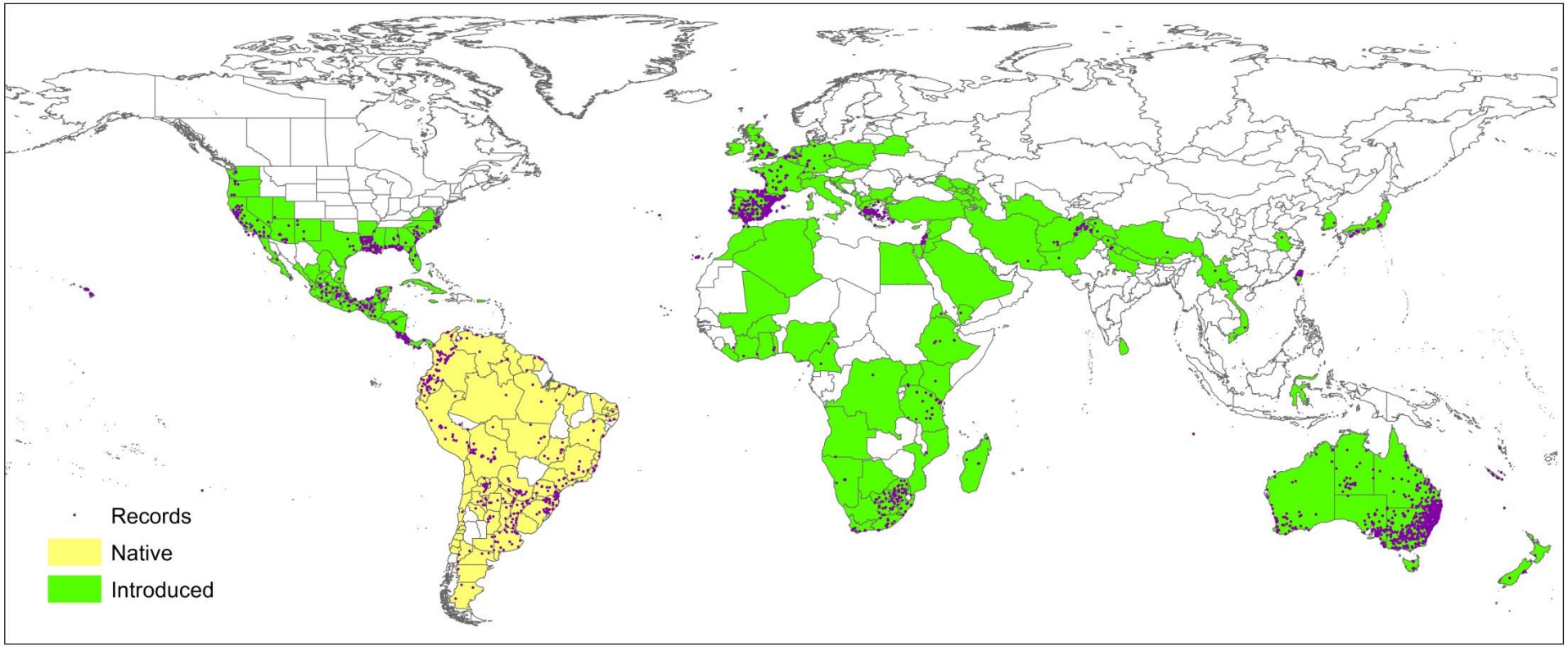
642 [a]Parameters without units are a dimensionless index of plant available soil moisture
643 scaled from 0 (oven dry) to 1.0 (field capacity). See Sutherst et al. (2007) for a
644 detailed description of parameters.
645
646

647 **Figure Captions**

648

649 Fig. 1. World distribution of *Conyza bonariensis*. Distribution records are shown as
650 dots. Regions where *C. bonariensis* has been recorded are shown by the coloured
651 areas either for the native or introduced distributions. Note only readily accessible
652 data sources were used to develop the map and the full range of *C. bonariensis* is
653 likely to include more regions than those indicated on the map.
654 Fig. 2. Development of seed germination per day (A), increase in plant dry weight (B)
655 and seedling longevity (C) (± S.E.) in *Conyza bonariensis* under different
656 temperature regimes (controlled temperature chambers (▲) and glasshouse (○).
657 Fig. 3. Cumulative seed germination of *Conyza bonariensis* under different
658 temperature regimes (GH = glasshouse) showing germination during the first two
659 weeks and the delayed germination occurring after 60 days.
660 Fig. 4. Projected world distribution of *Conyza bonariensis* as shown by the
661 Ecoclimatic Index (EI). CLIMEX climatic suitability as shown by the Ecoclimatic
662 Index (EI) is indicated by the changing colour scale: Unsuitable (EI = 0), Marginal
663 (EI = 1-30), Suitable (EI = 31-60), Optimal (EI > 60).
664 Fig. 5. South America and Australia showing Co*nyza bonariensis* records, projected
665 distribution of *C. bonariensis*. CLIMEX climatic suitability as shown by the
666 Ecoclimatic Index (EI) is indicated by the changing colour scale: Unsuitable (EI =
667 0), Marginal (EI = 1-30), Suitable (EI = 31-60), Optimal (EI > 60).Fig. 6. South
668 America and Australia showing *Conyza bonariensis* records, projected optimal
669 distribution of *C. bonariensis* (EI > 30, see Fig. 5), and projected increase or
670 decrease in EI for a hypothetical biological control agent (HBCA – cold)
671 developing at temperatures colder than that of *Conyza bonariensis.*

672 Fig. 7. South America and Australia showing *Conyza bonariensis* records, projected
673 optimal distribution of *C. bonariensis* (EI > 30, see Fig. 5), and projected increase
674 or decrease in EI for a hypothetical biological control agent (HBCA – hot)
675 developing at a temperature hotter than that suitable for *Conyza bonariensis.*



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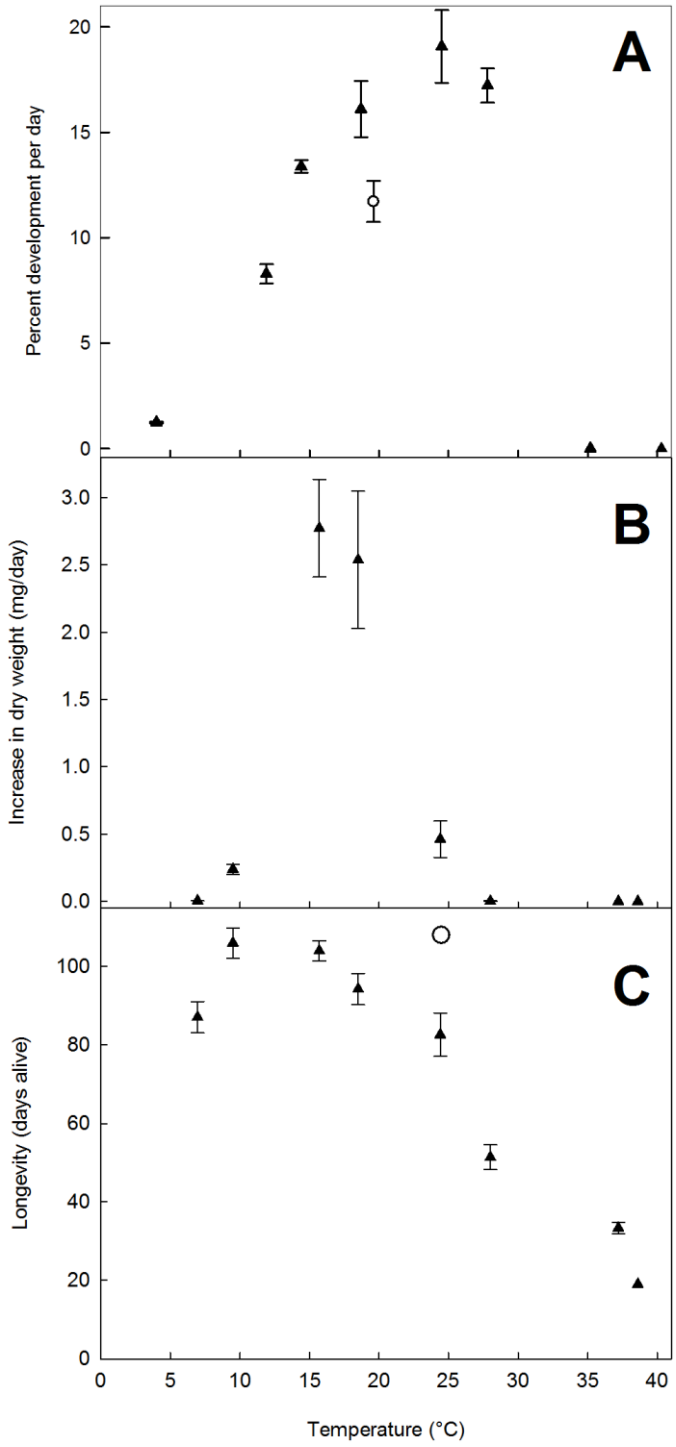
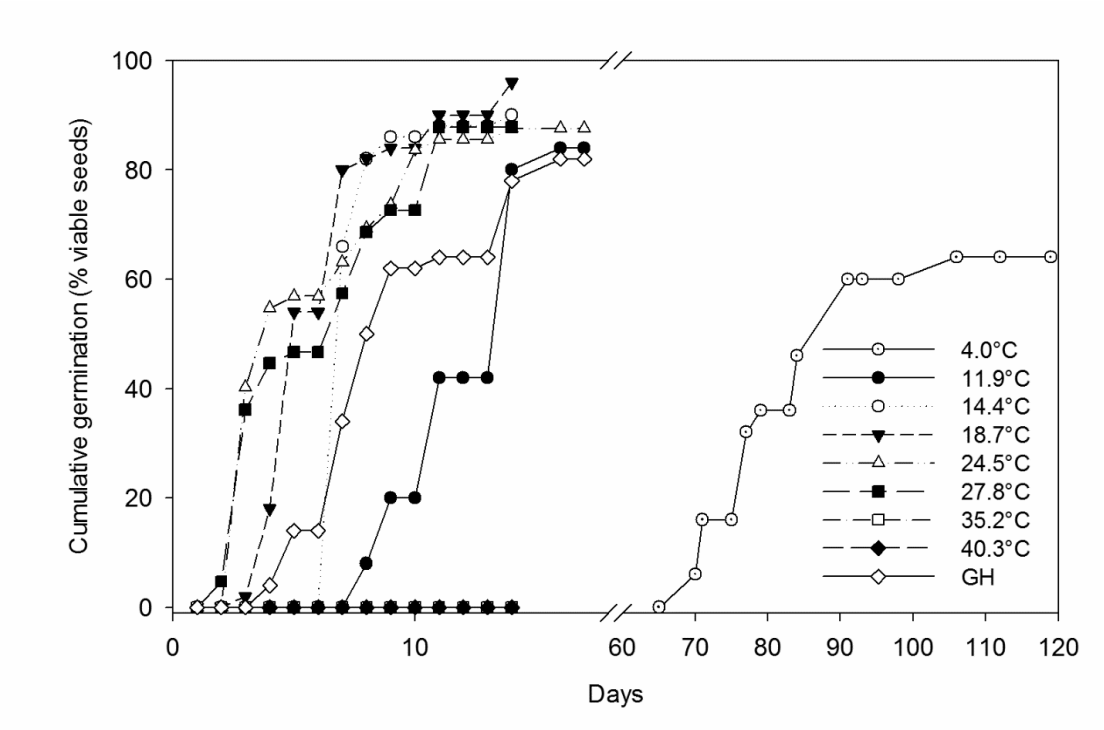


680

681 Fig. 2. Influence of different temperature regimes on developmental rates (time from
682 planting to time of germination) of seeds (A), and rates of biomass accumulation (B)
683 and longevity of seedlings (C) (± S.E.) in *Conyza bonariensis* (controlled temperature
684 chambers (▲) and glasshouse (○)).



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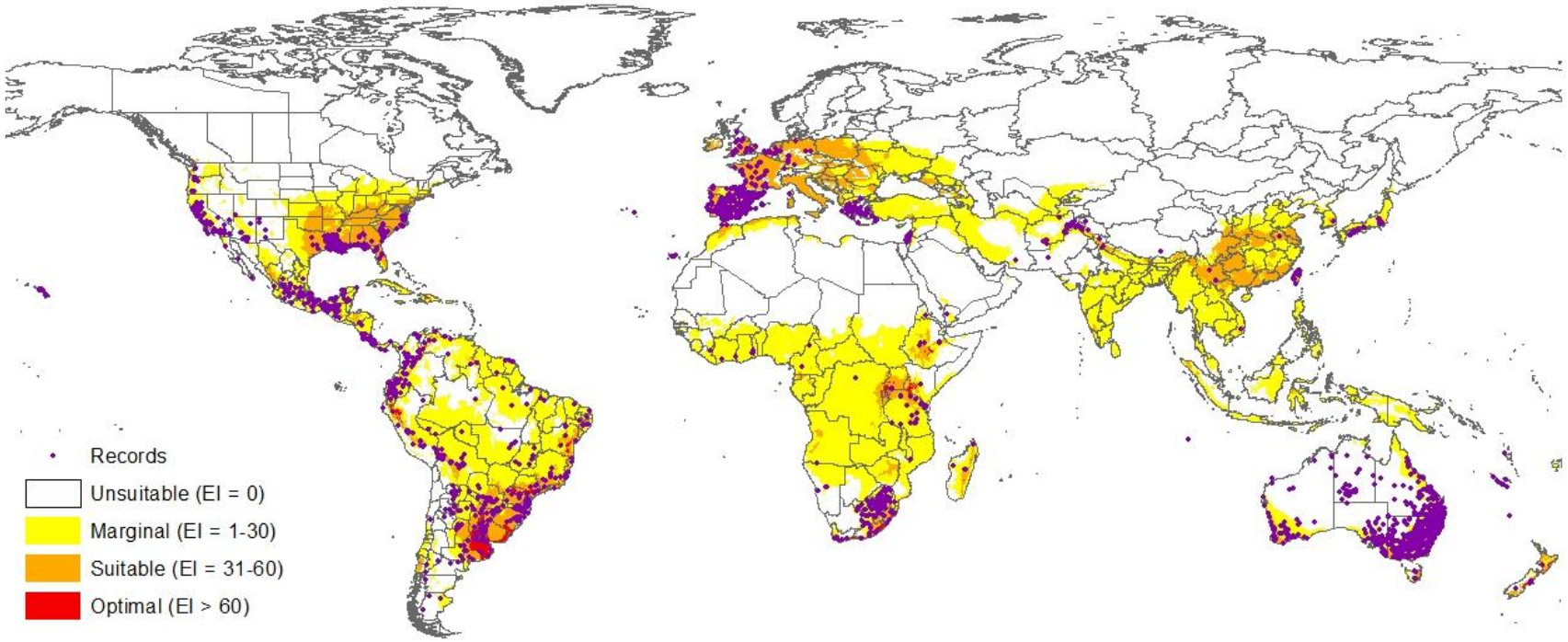

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693 Fig. 4. Projected world distribution of *Conyza bonariensis* as shown by the Ecoclimatic Index (EI). CLIMEX climatic suitability as shown by the

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695 60).

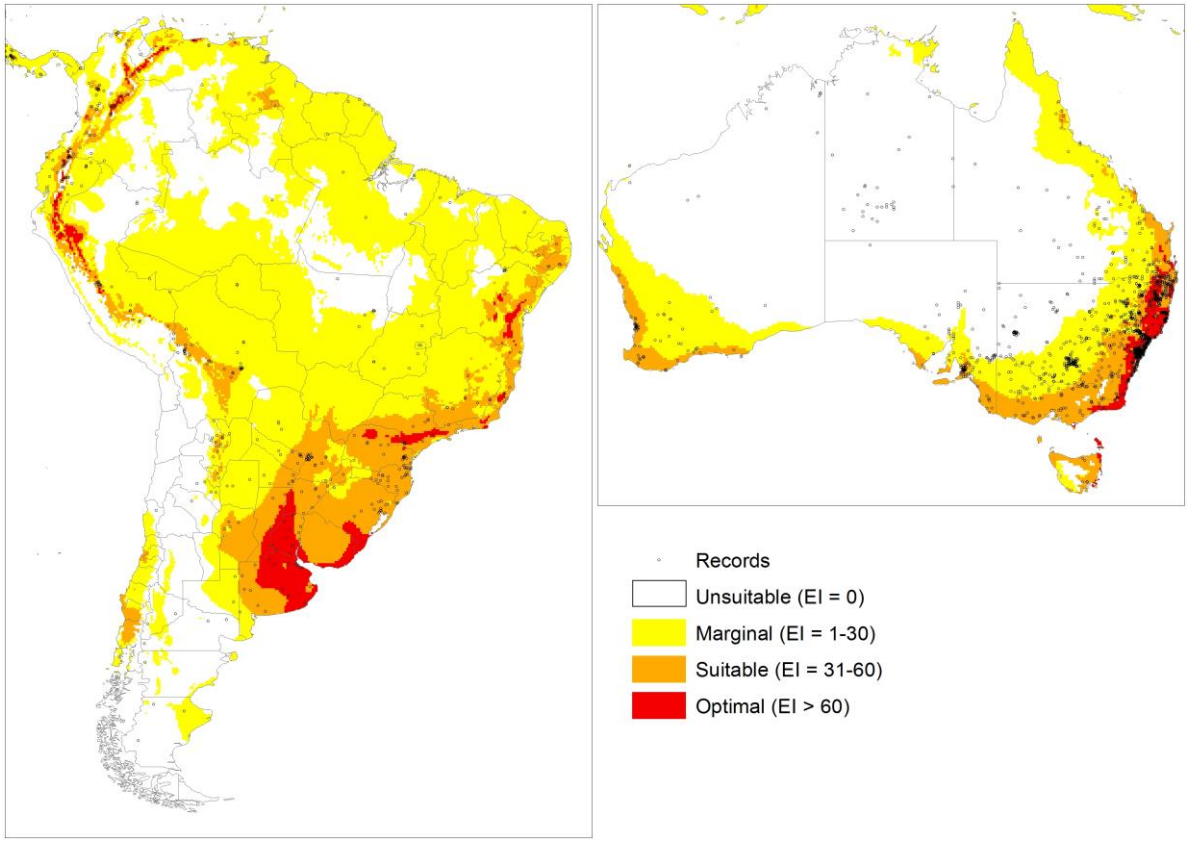

Fig. 5. South America and Australia showing Co*nyza bonariensis* records, projected distribution of *C. bonariensis*. CLIMEX climatic suitability as shown by the Ecoclimatic Index (EI) is indicated by the changing colour scale: Unsuitable (EI = 0), Marginal (EI = 1-30), Suitable (EI = 31-60), Optimal (EI > 60).

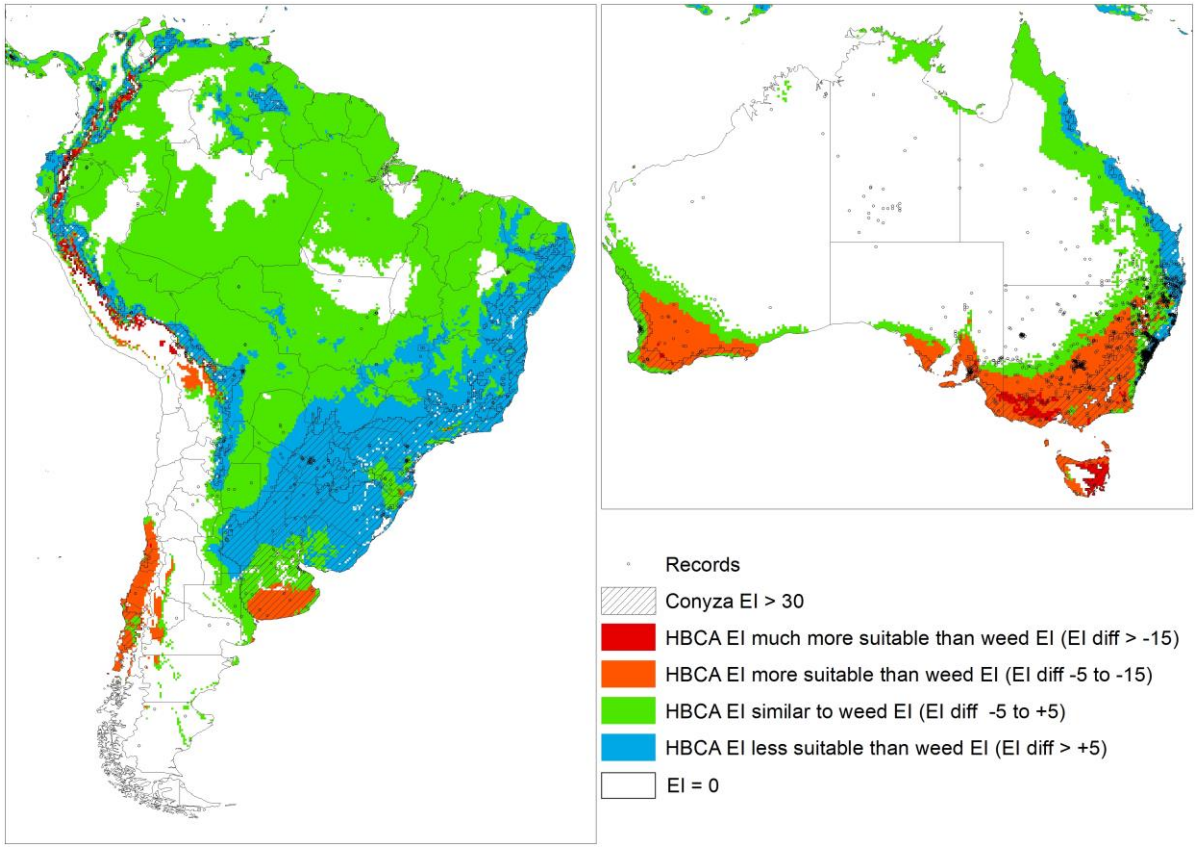

Fig. 6. South America and Australia showing *Conyza bonariensis* records, projected optimal distribution of *C. bonariensis* (EI > 30, see Fig. 5), and projected projected increase or decrease in EI for a hypothetical biological control agent (HBCA – cold) developing at temperatures colder than that of *C. bonariensis.*

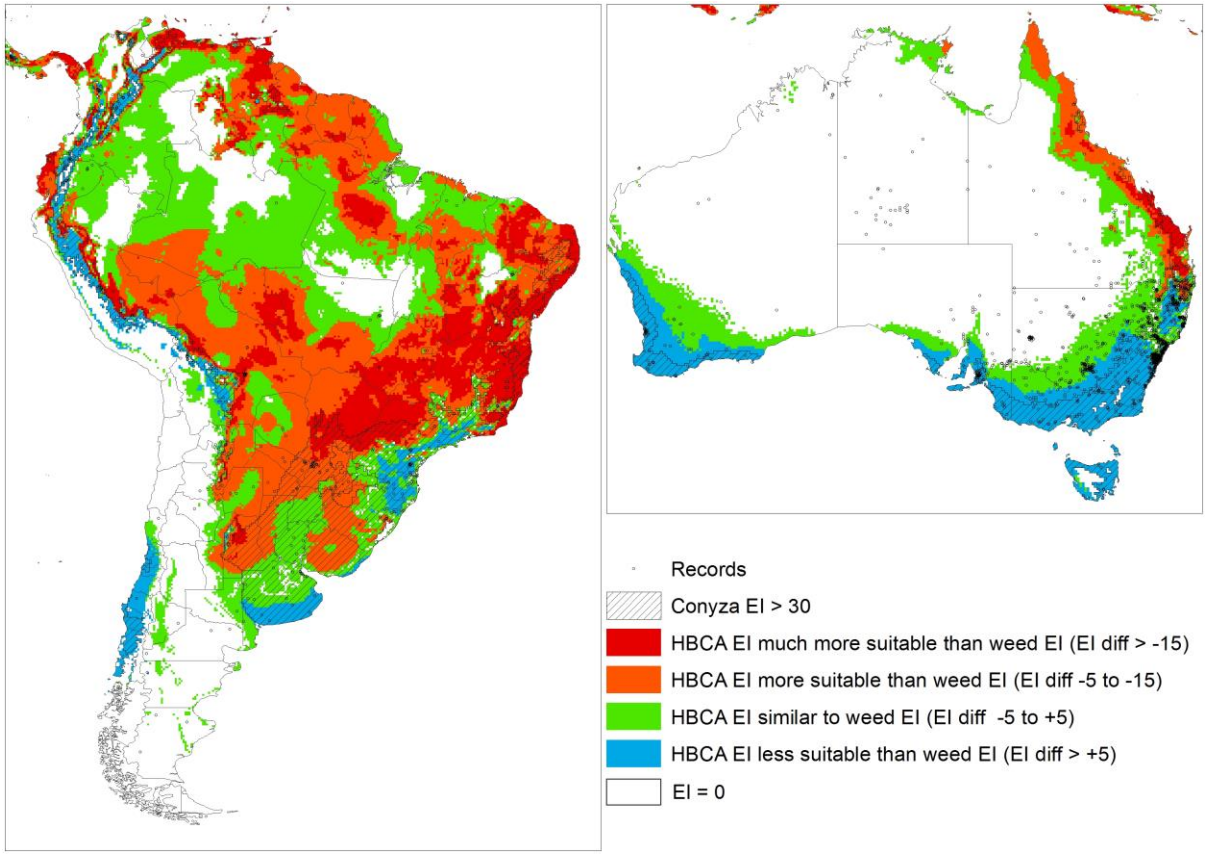

Fig. 7. South America and Australia showing *Conyza bonariensis* records, projected optimal distribution of *C. bonariensis* (EI > 30, see Fig. 5), and projected increase or decrease in EI for a hypothetical biological control agent (HBCA – hot) developing at a temperature hotter than that suitable for *C. bonariensis.*