Fire effects on plant reproductive fitness vary among individuals reflecting pollination-dependent mechanisms

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ABSTRACT

Premise

Fire induces flowering in many plant species worldwide, potentially improving reproductive fitness via greater availability of resources, as evident in flowering effort, and improved pollination outcomes, as evident by seed set. Post-fire increases in flowering synchrony, thus mating opportunities, may improve pollination. However, few studies evaluate fire effects on multiple components of fitness. Consequently, the magnitude and mechanism of fire effects on reproductive fitness remain unclear.

Methods

Over multiple years and prescribed burns in a prairie preserve, we counted flowering stems, flowers, fruits, and seeds of three prairie perennials*, Echinacea angustifolia*, *Liatris aspera*, and *Solidago speciosa*. We used aster life-history models to assess how fire and mating opportunities influenced annual maternal fitness and its components for individual plants.

Key Results

In *Echinacea* and *Liatris* but not *Solidago*: fire increased head counts, and both fire and mating opportunities increased maternal fitness. Burned *Echinacea* and *Liatris* plants with many flower heads produced many seeds despite low seed set (fertilization rates). In contrast, plants with an average number of flower heads only had high seed set and produced many seeds when mating opportunities were abundant.

Conclusions

Fire increased annual reproductive fitness via resource- and pollination-dependent mechanisms in *Echinacea* and *Liatris* but did not affect *Solidago* fitness. The consistent

relationship between synchrony and seed set implies temporal mating opportunities play an important role in pollination. While fire promotes flowering for many plant species, our results reveal that even closely related species exhibit differential responses to fire, which could impact the broader plant community.

KEYWORDS: aster model; components of fitness; flowering synchrony; grassland; herbaceous perennial; mating opportunities; prescribed burn; pollination; prairie; seed production;

INTRODUCTION

Fire influences the reproduction of herbaceous plants in fire-prone habitats worldwide (Ehrenreich and Aikman, 1963; Lamont and Downes, 2011; Pyke, 2017; Wagenius et al., 2020; Zirondi et al., 2021; Richardson and Wagenius, 2022). Fire stimulates flowering of many perennial species, increasing both the density of flowering plants and reproductive effort per flowering plant (Hulbert, 1988; Hartnett, 1990, 1991). However, the extent to which fire affects total annual maternal fitness, i.e., total seeds produced per plant per year, and the underlying mechanisms that influence fitness, remain unclear. Total annual maternal fitness, hereafter total reproductive fitness, is constrained by the number of stems, flowers, and fruits, as well as successful pollination. Because fire has the potential to both boost resources (Hulbert, 1988) and alter pollination outcomes (Ne'eman et al., 2000; García et al., 2018; LoPresti et al., 2018; Wagenius et al., 2020; Richardson and Wagenius, 2022), fire may affect total reproductive fitness via both resource-dependent mechanisms and pollination-dependent mechanisms. While fires can have a variety of negative impacts on plants and pollinators (Ne'eman et al., 2000; Gornish, 2013; Carbone and Aguilar, 2017, 2021; LoPresti et al.,

2018), fires may have positive effects on plant communities, particularly in fire-prone grasslands with fire-stimulated flowering (García et al., 2016; Fidelis and Zirondi, 2021; Morris et al., 2021; Richardson and Wagenius, 2022). Thus, fire and fire-stimulated flowering are globally relevant phenomena, yet the frequency of fires is changing in grasslands around the world, highlighting the importance of understanding the mechanistic basis by which fire influences reproductive outcomes of grassland plants.

Fire may improve total reproductive fitness of plants by influencing reproductive effort, pollination, or both. First, fire affects resource availability by increasing light and freeing nitrogen in prairies leading to increased flowering effort in some but not all grass species (Hulbert, 1988). If resource availability drives total reproductive fitness for most species in the context of fire-prone grasslands, we expect greater mean annual seed production for populations in burned areas compared to unburned areas. We would also expect all components of reproductive fitness dependent on resource allocation, including the counts of flowering stems, flowers, and fruits, to be higher for all burned plants. Furthermore, we expect most species, and individuals within species, to respond similarly to a boost in resources leading to low variation of allocation to reproductive structures among individuals after fire. Fire can increase flowering rates and reproductive effort (Ehrenreich and Aikman, 1963; Pemble et al., 1981; Hulbert, 1988; Hartnett, 1990, 1991; Vickery, 2002b; a; Pilon et al., 2018; Wagenius et al., 2020; Zirondi et al., 2021; Richardson and Wagenius, 2022). However, very little research examines how fire relates to variation in individual components of reproductive fitness, obscuring the underlying mechanism.

Independent of increasing resource availability, fire may also improve total reproductive fitness in plants by reducing pollen limitation. Whereas resource availability may affect the count of flowering stems, flowers, fruits, and seeds, pollination is expected to affect only the trait related to pollination, the ratio of fruits that become seeds, hereafter, seed set. Pollen limitation is expected to reduce reproductive fitness in contexts where pollination is uncertain (Haig and Westoby, 1988; Burd, 2008). Poor pollination outcomes due to limited mating opportunities are more likely for species persisting in small populations and those that only reproduce sexually, flower infrequently across years, and are self-incompatible (DeMauro, 1993; De Nettancourt, 1997; Davis et al., 2004; Wagenius et al., 2007; Waananen et al., 2018). Total reproductive fitness in these plants can therefore be mate-limited despite pollinator visitation (Wagenius and Lyon, 2010; Richardson et al., 2021). Because many prairie species are self-incompatible, flower infrequently across years, persist in small, fragmented habitats, and rely on sexual reproduction, they are therefore vulnerable to reproductive failure caused by mate-limitation. Previous studies have posited that fire improves reproduction in prairie plants by stimulating more plants to flower thus increasing the likelihood of successful pollination (Wagenius et al., 2020; Richardson and Wagenius, 2022).

Evidence that fire increases the proportion of ovules that develop seeds, i.e. seed set, by synchronizing flowering time and increasing availability of mates comes from two studies, a 20-year study of *Echinacea angustifolia* (Wagenius et al., 2020) and a 3-year study of *Liatris aspera* and *Solidago speciosa* (Richardson and Wagenius, 2022). In both *E. angustifolia* and *L. aspera,* fire increased within- and among-year flowering synchrony leading to a higher proportion of fruits with seeds in burned plants compared to unburned

plants. However, fire did not increase flowering rates or improve seed set in *S. speciosa*, despite synchronizing within-year flowering time (Richardson and Wagenius, 2022). Prior studies on *E. angustifolia* showed that pollinators are prevalent and that co-flowering heterospecific plants facilitated pollinator visitation although heterospecific pollen interference was rare (Richardson et al., 2021). Nonetheless, pollination failure is well documented in *E. angustifolia* and increases when plants are isolated in either time or space from co-flowering mates (Wagenius, 2004; Ison et al., 2014; Richardson et al., 2021). While these studies have shown an important relationship between pollen limitation and seed set at the population-level, understanding the mechanism by which fire affects seed production requires examining multiple components of reproductive fitness and examining individual variation in fitness. If fire affects fitness via pollination-dependent mechanisms, then we expect variation in individual fitness to correlate with mating opportunities such as the temporal or spatial proximity of flowering conspecifics. Determining these relationships relies on quantifying multiple components of reproductive fitness.

Investigating multiple components of fitness in a single analysis presents several challenges (Shaw et al., 2008). First, individual components of fitness are not independent (Fig. 1). Total seed production, for example, is constrained by the number of fruits that were produced, which is in turn constrained by the number of flowers and flowering stems. Second, the resulting distribution of total reproductive fitness, rarely conforms to conventional statistical distributions; few individuals have remarkably high total reproductive fitness, many individuals fail to produce any seeds, and some individuals have intermediate fitness. These fitness distributions are common among

plants with modular growth habits. Because of these challenges, some researchers examine each fitness component in isolation, obtaining conditional estimates of fitness such as the number of fruits produced contingent upon flower production. Conditional estimates improve understanding of each fitness component alone but do not reveal how those components together contribute to total reproductive fitness. Aster life history models are a class of statistical models that handle these complex data in a single analysis (Geyer et al., 2007; Shaw et al., 2008). Aster models account for the directional and dependent nature of fitness components and allow us to make both conditional estimates of fitness for each component as well as unconditional estimates of total reproductive fitness from a single model. Therefore, to jointly model the components of reproductive fitness as they relate to fire and mating opportunities, we utilized aster life-history models.

Fire-stimulated flowering is prevalent across fire-dependent ecosystems worldwide yet the mechanisms by which fire affects total reproductive fitness are not well understood. Previous empirical studies have largely focused on how fire affects reproductive effort while fire effects on pollination and seed production have received comparably little attention (Hartnett, 1990; Vickery, 2002b; a; Pilon et al., 2018; Zirondi et al., 2021, but see Wagenius et al., 2020). Furthermore, plant species exhibit different reproductive responses to fire (Ehrenreich and Aikman, 1963; Pemble et al., 1981). Quantifying differences in fire effects among species and how these differential responses influence total reproductive fitness will improve our understanding mechanisms by which fire influences plant reproduction. We investigated fire effects on plant reproduction in three perennial, iteroparous plant species within a regularly burned

prairie remnant to elucidate how resource-dependent and pollination-dependent mechanisms influenced reproductive fitness. We tracked individual flowering time, and counted flowering stems, flowers, fruits, and seeds of three perennial tallgrass prairie species. We sampled 350 *E. angustifolia* individuals across seven years, as well as 223 *L. aspera* and 231 *S. speciosa* individuals across three years. By assessing total reproductive fitness and its components, we disentangle the extent to which fire affects reproduction in grassland plants via resource- and pollination-dependent mechanisms. We hypothesize that fire stimulates plants to produce more flowering heads. We also hypothesize that fires improve mating opportunities for plants by synchronizing flowering time, leading to better pollination outcomes. Finally, we compare reproductive responses to fire among species. In particular, based on prior work in this system, we expect similar and positive responses to fire in *E. angustifolia* and *L. aspera* and a lack of response in *S. speciosa* (Richardson and Wagenius 2021, Wagenius et al 2020). Our results broaden understanding of the effects of fire on reproductive responses in plants in fire-suppressed systems.

MATERIALS AND METHODS

***Study species*—**

To investigate the mechanisms by which fire affects reproductive fitness in grassland forbs, we selected three study species within the Asteraceae family: *Echinacea angustifolia* DC., *Liatris aspera* Michx. and *Solidago speciosa* Nutt. These focal species share reproductive traits common to many plants inhabiting tallgrass prairie. Species within Asteraceae (the most speciose plant family worldwide and in the prairie) also exhibit several reproductive characteristics that facilitate inferences about the differential effects of resource- and pollination-dependent mechanisms. By comparing the differential

effects of these mechanisms in three members of Asteraceae, we gain insights into how fire influence plant reproduction via resource- and pollination-dependent mechanisms as well as the degree to which fire effects on these different mechanisms vary among species with similar reproductive traits and evolutionary histories.

All three species are long-lived and non-clonal such that individuals are easy to identify, and they must reproduce by seed. Furthermore, these species are widespread in prairie remnants rather than rare, and are all sporophytically self-incompatible (Levin, 1967; Gross and Werner, 1983; Wagenius, 2004). Each species produces composite heads (capitula) that contain uniovulate florets in which the embryo is provisioned after pollination. Thus, each floret on a head produces a single fruit (achene) that may or may not contain a seed. Generalist native pollinators visit all three species, primarily native ground-nesting bees but also bumblebees and butterflies, all of which are prevalent at the study site (Wagenius and Lyon, 2010; Manion, 2021). The frequency of reproductive bouts varies among these three species, with *E. angustifolia* and *L. aspera* rarely flowering two years in a row, and *S. speciosa* flowering nearly annually (Richardson and Wagenius, 2022). Each summer plants re-grow from their perennial root, which accumulate resources each year. Both *E. angustifolia* and *S. speciosa* have taproots whereas *L. aspera* has corms. When initiating new aboveground growth each year, all three species either produce flowering stems or remain in a vegetative state. *Liatris aspera* and *E. angustifolia* produce one or more rosettes annually, each rosette is either cauline, with the potential to flower, or acaulescent, strictly vegetative. *Solidago speciosa* produces only cauline rosettes. When flowering, *E. angustifolia* individuals typically generate one to several flowering stems with one head per stem, whereas *L. aspera* plants

generate one to four stems with 10–30 heads each, and *S. speciosa* plants generate one to three stems, but occasionally up to 40, with 10–250 flowering heads per stem.

Study site—

Our study site is Staffanson Prairie Preserve, a 45 ha tallgrass prairie remnant in Douglas County, Minnesota, USA, managed by the Nature Conservancy since 1972. Most of the site was never plowed but was hayed between 1930 and 1980. Agricultural fields of corn and soybeans now surround the preserve and dominate the landscape that was once continuous tallgrass prairie. Prescribed burns have been used to manage the preserve and have been conducted on an alternating four-year cycle for decades, such that only half of the preserve burns in any given burn year and every other year no burn occurs (see Table 1). Each unique combination of management unit and year comprises a mating scene (e.g. east unit in 2012) as in prior studies of these species at this site (Wagenius et al., 2020; Richardson and Wagenius, 2022). Here, a mating scene characterizes individual mating opportunities by quantifying the overlap in flowering time of each individual plant with all other plants in the scene. In each year except 2017, there is one burned mating scene and one unburned mating scene. In 2017 neither scene was burned. Burns are conducted while all three study species are dormant. Fires combust the dry aboveground biomass and leaf litter without killing perennial prairie plants. In our three study species, the effects of fire are visible during the subsequent flowering season (Wagenius et al., 2020; Richardson and Wagenius, 2022). In this study, plants that were observed following a burn are called “burned plants” and those plants that were not burned during the prior dormant season are called “unburned plants.”

Field methods—

Assessing individual components of reproductive fitness— To assess the effects of fire and mating opportunities on multiple components of reproductive fitness in three prairie perennials, we established transects in both management units of the study site and selected plants for observation. For *E. angustifolia,* we have two permanent mapped belt transects, one in each management unit of the prairie preserve (0.449 ha in the west unit and 0.997 ha in the east unit), within which we censused all flowering individuals each summer between 1996 and 2016. All flowering *E. angustifolia* plants are included as individuals in this study; plants that did not flower are not included in this study. Similarly, for *L. aspera* and *S. speciosa,* we laid out two belt transects (0.016 ha in both the east and west units) along a randomly selected latitude line and identified all flowering plants within those transects from 2016 to 2018. Flowering densities were higher for *L. aspera* and *S. speciosa* compared to *E. angustifolia* so we randomly selected flowering individuals from among all flowering individuals within the transect to be included in the study. Additional details of the field sampling for *E. angustifolia* can be found in Wagenius et al (2020), whereas details for *L. aspera* and *S. speciosa* can be found in Richardson & Wagenius (2022). The total number of individuals included in the study for each species in each year are found in Table 2.

To measure the components of reproductive fitness in each individual, we counted flowering stems, heads, fruits, and seeds with slight differences among study species and years as described in Table 2. For example, for *E. angustifolia,* each flowering stem almost always has a single head so stem counts and head counts are often redundant. Obtaining detailed fruit counts of *L. aspera* was extremely laborious and thus only occurred in 2018. Similarly, obtaining both head and fruit counts was extremely laborious

for *S. speciosa* and thus only obtained in 2018. Detailed protocols for counting fruits and seeds and assessing seed set of each species are described in Appendix 1. We should note that seed set, i.e., the proportion of uniovulate fruits that contain an embryo, was obtained via inspecting x-ray images rather than by germinating seeds. We visualized the spread of observed data for each fitness component of each species in each mating scene by plotting means and standard errors.

Quantifying mating opportunities— To quantify mating opportunities of individual plants, we calculated individual flowering synchrony, which relies on precise records of first and last day of flowering for all individuals in the mating scene. Thus, we visited all plants every two days, recorded the first day of flowering as the day that anthers first shed pollen, and the last day of flowering as the latest day that a plant shed pollen (detailed methods in Wagenius et al., 2020 and Richardson and Wagenius, 2022, for *E. angustifolia*, and *L. aspera* and *S. speciosa*, respectively.)

We calculated Augspurger's (1983) index of synchrony in R package mateable (Wagenius et al., 2016) as a proxy for temporal mate-availability because it is commonly used in plant phenology studies with similar data structure to ours (Ollerton and Lack, 1998; Rodríguez-Pérez and Traveset, 2016; Waananen et al., 2018; Wagenius et al., 2020; Richardson and Wagenius, 2022). Individual flowering synchrony is an index of the degree to which the timing of flowering of an individual overlaps temporally with the flowering of all other individuals in the mating scene. For an individual plant that flowered at a time when no other plants in the mating scene were flowering, individual flowering synchrony is zero, indicating no mating opportunities. In contrast, the flowering synchrony for an individual is one for an individual having a flowering period

during which all other plants in the mating scene also flowered. Augspurger's method is not the only measure of synchrony (reviewed in Elzinga et al., (2007) and other metrics may be preferable to Augspurger's method when flowering effort within individuals varies throughout the individual's flowering period. Given the flowering pattern in our study species, we expect that the number of florets receptive to pollination of an individual will only slightly increase near the individual's mid-flowering time such that many-headed plants with long flowering duration will have a low proportion of their florets receptive to pollination during peak flowering, when successful mating is most likely. Thus, the use of Augspurger's index of individual flowering synchrony should be appropriate for this study.

Analysis—

Constructing the aster model graphical response— The central aim of this study is to compare how both fire and flowering synchrony, characteristics of the mating scene, differentially relate to total reproductive fitness in these three species. Fire is associated with higher flowering synchrony and increases in seed set, or the proportion of fruits that produce seeds, in *E. angustifolia* and *L. aspera,* but not *S. speciosa* (Wagenius et al., 2020; Richardson and Wagenius, 2022). However, we do not know the relationships between fire, temporal flowering synchrony, and total counts of flowering stems, heads, fruits, and seeds. To estimate the joint effect of fire and flowering synchrony on components of fitness, we used aster life history models and R package 'aster' (Geyer, 2021). Aster models jointly model all fitness components as a graphical response variable in one analysis, such that seed production is dependent on the count of flowering stems, heads, and fruits and each of those fitness components is associated with an appropriate

probability distribution. For example, counts of heads and fruits are modeled with Poisson distributions whereas the proportion of fruits that contain seeds are modeled with a Bernoulli distribution in this analysis (Figure 1). Both the Poisson and Bernoulli distributions are appropriate probability distributions for modeling, respectively, count and binary variables. In *S. speciosa* and *L. aspera* the graphical response variable is: flowering stem count → head count → fruit count → seed count. In *E. angustifolia* plants nearly always have only one head per stem, so we remove the stem count node. Species were modeled separately because of underlying differences in sampling years and graphical models. Because counts of fruits were not recorded in the 2016 and 2017 data for *L. aspera* and *S. speciosa*, we began by constructing aster models for these species only using the complete 2018 data. In these null unconditional aster models the response variable is the entire graphical model as described above.

Predictors— To determine how fire and flowering synchrony relate to reproductive fitness, while accounting for the effect of management unit and year, the following predictors were used for all species: synchrony, burn (a factor with 0 for no burn and 1 for burn), and an interaction term (burn X synchrony). For *E. angustifolia,* we used additional predictors: management unit (categorical predictor indicating the presence of an individual in the east or west unit of the preserve), year, and an interaction term (burn X year). We did not include unit, year, or the burn X year interaction terms as predictors in models for *L. aspera* and *S. speciosa* because each year was modeled separately, and all model selection as described below was performed using only the models that fit the complete 2018 data. Table 3 lists all predictors.

Aster models with predictors— First, we constructed unconditional aster models for each species such that predictors could influence the entire graphical response variable, by specifying

dependence of the terminal node of the graphical response variable (i.e., seed count) on the predictors. Next, to understand how predictors relate to individual components of fitness in each species, we constructed unconditional aster models and allowed each fitness component (i.e., stem count, head count, or fruit count) to vary with predictors as well as with the terminal node of the graphical response variable, 'seed count'. This approach reveals additional relationships between fire, flowering synchrony, and components of reproductive fitness other than total seed production. Finally, for each species we constructed a selection of biologically interpretable and statistically reasonable models with combinations of predictors and interaction terms (see Appendix S1, Table S1 (see the Supplementary Data with this article) for all *E. angustifolia* models, and Appendix S1, Table S2 for all *L. aspera* and *S. speciosa* models).

Model selection— We used AIC to compare all models and identify the best model, i.e., the model with the lowest AIC, for each study species. Because we were missing fruit count data in 2016 and 2017 for *L. aspera* and *S. speciosa*, we selected the best model for each species using only 2018 data, which was not missing any values. We then applied those best models for *L. aspera* and *S. speciosa* to the 2016 and 2017 data with simulated values of fruit count. To create datasets with simulated values for fruit count, we followed a multiple imputation approach (Rubin, 1987) using the data we observed in 2018 as a distribution from which to resample values. We created 250

datasets for both *L. aspera* and *S. speciosa* with simulated fruit count values for 2016 and 2017 and then fit the best models to all 250 of those simulated datasets for each species.

To visualize the relationship between fire, flowering synchrony, and each fitness component in each species, we constructed a figure to show predicted values, both conditional and unconditional (Figure 1), from the best model for hypothetical individuals of each species within the range of observed values for flowering synchrony. All analyses were conducted in R (R Core Team, 2020).

Limitations—

Although we endeavor to understand how fire affects plants, we note that certain assumptions of our analytical approach are not met. First, mating scenes sampled in different years are not truly independent because they are sampled from the same locations (i.e., the same two management units were sampled each year). Second, the individual plants we sampled are not truly independent and are thus pseudoreplicated (Hurlbert, 1984). Thus, burn effects may be confounded with factors associated with a given place or a given time. Nonetheless, if results reveal consistent patterns across both space and time, burn effects would be the most parsimonious explanation.

RESULTS

Echinacea angustifolia—

In *Echinacea angustifolia,* total reproductive fitness (Fig. 2), i.e., total seed production per plant, was two times higher on average for burned plants compared to unburned plants (see means in Fig. 2g, model selection results in Appendix S1, Table S3, and means predicted by the model in Fig. 3g). Two components of fitness, mean seed set and mean head count, were around 50% higher for burned plants than unburned plants

(means in Fig. 2a and 2d, means predicted by the model in Fig. 3a and 3d). In contrast, mean fruit count per head did not differ between burned and unburned plants (Fig. S1).

In the best model for *E. angustifolia* (Appendix S1, Table S3), increased total reproductive fitness was associated with fire ($p < 0.0001$) and flowering synchrony ($p < 0.0001$) after accounting for the effect of management unit ($p < 0.0001$) and year ($p < 0.0001$). Head count was also associated with fire and flowering synchrony in the best model (fire X year, $p = 0.0066$; synchrony, $p < 0.0001$). We found no evidence that the effects of fire and flowering synchrony on fruit count differed from the effects of fire and flowering synchrony on total reproductive fitness (i.e., none of the aster models in Appendix S1, Table S3 with low AIC values included interactions between predictors and the fruit count node of the graphical response). Here, we clarify that we do not expect flowering synchrony to have a biological effect on fruit count, since fruit count in these species is the same as floret count which is determined before the plant begins flowering.

Because head count and seed production are both related to flowering synchrony, understanding the relationship between synchrony and fitness is best understood by examining Fig. 3a, 3d, and 3g together. Plants with high and low synchrony tend to have higher head counts than those with intermediate synchrony, particularly for burned plants (Fig. 3a). Plants with higher flowering synchrony had higher seed set regardless of fire (Fig. 3d). To facilitate understanding, we describe hypothetical individuals in each panel of Fig. 3. In Fig. 3a burned plants with median head counts exhibit a range of synchrony, but that those with high synchrony tend to have high seed set (Fig. 3d.) and subsequently many predicted seeds per plant (Fig. 3g). Plants with very high head counts in Fig. 3a,

including the burned plant with 6 heads, have low synchrony and low seed set (Fig. 3d) nonetheless are predicted to produce many seeds (Fig. 3g).

***Liatris aspera*—**

In *L. aspera*, similarly to *E. angustifolia,* fire and flowering synchrony interacted to more than double total reproductive fitness in burned plants compared to unburned plants (see Fig. 2h for means, Fig. 3h for means predicted by the model, and Appendix S1, Table S4 for results of model selection, burn X synchrony, $p = 0.009$). Components of reproductive fitness including mean fruit count per head (Appendix S2, Fig. S1) and mean stem count (Appendix S2, Fig. S2) did not differ between burned and unburned plants. However, in burned compared to unburned plants, mean seed set was on average 45% higher (Fig. 2e for means, Fig. 3e for means predicted by the model), and mean head count was 55% higher (Fig. 2b for means and Fig. 3b for means predicted by the model).

In the best model, both head count and total reproductive fitness varied with fire and flowering synchrony of *L. aspera* (burn, $p < 0.0001$; synchrony, $p < 0.0001$, Appendix S1, Table S4). Neither stem count nor fruit count varied with fire or flowering synchrony in a way that deviated from how total reproductive fitness varied with fire and flowering synchrony, as evidenced by high AIC value for models that contained interaction terms between stem count or fruit count, and fire or flowering synchrony (Appendix S1, Table S4).

Because both head count and total reproductive fitness varied with fire and synchrony of *L. aspera*, understanding the relationship between synchrony and fitness components is not straightforward. In examining Fig. 3b, we note that plants with the highest head counts were burned plants with low synchrony. Plants with average head

counts could have low, intermediate, or high synchrony. The relationship between synchrony and seed set was not obvious although burned plants had higher synchrony on average (Fig. 3e).

***Solidago speciosa*—**

In contrast to total reproductive fitness of *E. angustifolia* and *L. aspera*, total reproductive fitness of *S. speciosa* did not respond to fire, despite the best model including an interaction term between fire, flowering synchrony, and total reproductive fitness (burn X synchrony, $p = 0.027$, see Appendix S1, Table S5 for results of model selection). When we examined how fire and flowering synchrony related to components of total reproductive fitness of *S. speciosa*, we found that fire and synchrony varied with fruit count differently than with total reproductive fitness (fire, $p < 0.0001$ and synchrony, $p < 0.0001$). We gained no insight by including interactions of fire and flowering synchrony with stem count or head count, as evidenced by high AIC values for models including such interactions (Appendix S1, Table S5).

Despite an association between fire, flowering synchrony, total reproductive fitness, and fruit count according to our best model (Appendix S1, Table S5), fire and flowering synchrony neither increase or decrease mean fitness components, as shown by examination of means (Fig. 2c, f, i) and values predicted by the best model (Fig. 3c, f, i, model selection in Appendix S1, Table S5). The east unit of the preserve had consistently higher mean stem count per plant (Fig. 2c) which drove the observed difference in seeds per plant in 2018 (Fig. 2i). While mean stem count was consistently higher in the east unit, the medians were more similar across units (median stem count and 95% C. I. in east = 3 (2, 5), and west = 2 (1, 3)).

Comparing results among species (Table 4, Fig. 2, 3) reveals two distinct types of responses to fire. Both *E. angustifolia* and *L. aspera* showed similar and strong responses to fire, presumably via both resource- and pollination-dependent mechanisms, whereas *S. solidago* did not respond to fire.

DISCUSSION

Fire enhanced multiple components of total reproductive fitness, via presumably resource-dependent and pollination-dependent mechanisms in two of our three study species. However, variation among species, and among individuals tied to mating opportunities, calls into question the traditional view that fire effects on reproduction only reflect resource availability and resource-dependent mechanisms (Hulbert, 1988; Lamont and Downes, 2011). Our study highlights the importance of investigating the variation in multiple components of fitness at the individual-level for uncovering mechanisms driving fitness outcomes.

Fire in *E. angustifolia* and *L. aspera* affected total reproductive fitness through pollination-dependent mechanisms. In both species, burned compared to unburned plants had more available mates, i.e. higher temporal flowering synchrony, higher average seed set (Fig. 2d, e) as has been reported before (Waananen et al., 2018; Wagenius et al., 2020; Richardson and Wagenius, 2022), and higher average seed production (Fig. 2g, h). Results for *E. angustifolia* were consistent with the hypothesis that plants with more available mates produce more seeds because of improved pollination outcomes, as *E. angustifolia* plants with mean head counts and high flowering synchrony had high seed set regardless of fire (Fig. 3d). Additionally, burned *E. angustifolia* and *L. aspera* plants with the most flowering heads had low flowering synchrony and low seed set (Fig. 3a, b,

d, e), which is consistent with the hypothesis that mate-limitation reduces pollination success for self-incompatible species due to a mate-finding Allee effect (DeMauro, 1993; De Nettancourt, 1997; Davis et al., 2004; Wagenius et al., 2007). Our findings also align with the expectation that seed production in self-incompatible Asteraceae should be more sensitive to increased pollination than increased resources (Wagenius, 2004; Chamer et al., 2015; Tamburini et al., 2017; Richardson and Wagenius, 2022). *Solidago speciosa*'s lack of response to fire also aligns with this expectation because whatever boost of resources provided by fire did not affect seed production, and pollination outcomes in *S. speciosa* may be driven by processes unrelated to temporal synchrony and fire.

Increases in head counts and fruit counts after fire provided evidence that fire affected fitness via resource-dependent mechanisms in *E. angustifolia* and *L. aspera*. *Echinacea angustifolia* and *L. aspera* plants with many stems and heads yielded high total reproductive fitness even when pollination success (seed set) was low (Fig. 3 a, b, g, h), showing that allocating many resources to reproduction improved total reproductive fitness. Despite the relationship we detected whereby head counts in *E. angustifolia* and *L. aspera* increased with fire, we can't be sure that a post-fire boost in resources is responsible. A non-resource trigger may induce plants to invest a greater proportion of stored resources in flowering than they would in a year without the fire cue. For example, aspects of fire that may cue flowering could include exposure to smoke or heat. Such a strategy may increase reproductive returns. Future studies that manipulate resources directly could improve understanding of the degree to which fire improves reproductive fitness via a post-fire pulse in resources.

The differences in post-fire reproductive responses among species points to complex relationships between resource availability, allocation of resources to reproduction within and among years, and total reproductive fitness. Like our study, previous studies have shown interspecific variation in reproductive response to fire (Ehrenreich and Aikman, 1963; Pemble et al., 1981; Hulbert, 1988; Zirondi et al., 2021). There are multiple potential explanations for why reproductive response to fire differed among our study species. Species could have differing sensitivities or responses to fire itself, or they may differ in their responses to a post-fire boost in resources. One potential explanation for interspecific variation in reproductive response to fire is variation in flowering frequency. In iteroparous species, plants may flower annually or less frequently. The degree to which resource pulses stimulate reproduction in iteroparous species may depend on flowering frequency. In our study species, *S. speciosa* flowers nearly annually, whereas *E. angustifolia* and *L. aspera* rarely flower two years in a row (Waananen et al., 2018; Richardson and Wagenius, 2022). In addition, species may differ physiologically in response to a resource pulse, or they may differ in their competitive abilities, all of which could be modulated by fire. Our results clearly demonstrate that fire effects on reproductive fitness differ among species which has implications for plant communities after fires.

CONCLUSIONS

Our results reveal that fire increases annual maternal fitness in species where fire stimulates flowering, through both resource-dependent and pollination-dependent mechanisms. The variation we found among species and among individuals within

species indicates that resource pulses from fire do not simply translate into increased flowering and seed production. Future work in this system could focus on how variation in reproductive response to fire among species affects species interactions such as competitive hierarchies within the plant community as well as with organisms at higher trophic levels. Because of variation among the species that we observed in the extent of fire-stimulated flowering and the resulting boost to seed production, we also expect interactions with higher trophic levels, such as pollinators and seed predators, to be altered after fire. Our results also suggest that species that respond to fire by flowering may depend on fire to maintain seed production. Whereas our study species are relatively common in the study site, many other co-occurring native species are comparably rarer, and may be even more dependent on fire to maintain reproductive fitness, particularly if they exhibit fire-stimulated flowering and reproduce solely by seeds. We also highlight the value of assessing multiple components of fitness simultaneously, especially when the goal is to clarify mechanisms driving fitness outcomes. This work contributes to a better understanding of the effects of fire on reproduction of perennial plants, particularly in fire-prone regions where fires are suppressed. Our results may be less applicable to ecosystems experiencing increased fire frequency and severity due to climate change. Thus, grassland plants that flower in response to fire may be at risk if fires are suppressed; they may fail to invest enough effort into flowering, and the effort they invest may be wasted if mates are scarce and pollination fails.

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AUTHOR CONTRIBUTIONS

All authors contributed to the design of the experiment. L.K.R., S.W., and J.B. conducted the field experiment. S.W. and J.B. prepared the *E. angustifolia* data while L.K.R. prepared the *L. aspera* and *S. speciosa* data and conducted lab work associated with those species. D.J.E., R.G.S., and S.W. designed the analytical methods. D.J.E. and L.K.R analyzed the data. L.K.R. prepared all figures and wrote the manuscript. All authors contributed substantially to editing and proofing the manuscript.

DATA AVAILABILITY STATEMENT

The data and code used for the analyses in this manuscript are archived in Figshare, DOI: 10.6084/m9.figshare.22186480.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information section at the end of the article.

Appendix S1 Tables showing all fitted aster models and model selection tables from analyses for each study species.

Appendix S2 Supplemental figures of fitness components.

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TABLES

Table 1 Dates of prescribed burns since 1996 in the two management units at the preserve.

Year	Unit burned	Burn date
1996	West	29 April 1996
1998	East	30 April 1998
2001	West	11 May 2001
2003	East	28 April 2003
2007	West	2 November 2006
2009	East	25 April 2009
2012	West	21 May 2012
2014	East	27 April 2014
2016	West	15 May 2016
2018	East	3 May 2018

Table 2 Number of individuals as well as the types of data available for each species in each mating scene throughout the study.

Species	Year	Burn	# Individuals	Stems	Heads	Fruits	Seed set
E. angustifolia	1996	*No*	4	*Yes*	*Yes*	*Yes*	*Yes*
	1996	*Yes*	19	*Yes*	*Yes*	*Yes*	*Yes*
	1998	*No*	8	*Yes*	*Yes*	*Yes*	*Yes*
	1998	*Yes*	25	*Yes*	*Yes*	*Yes*	*Yes*
	2007	*No*	27	*Yes*	*Yes*	*Yes*	*Yes*
	2007	*Yes*	31	*Yes*	*Yes*	*Yes*	*Yes*
	2009	*No*	39	*Yes*	*Yes*	*Yes*	*Yes*
	2009	*Yes*	34	*Yes*	*Yes*	*Yes*	*Yes*
	2012	*No*	9	*Yes*	*Yes*	*Yes*	*Yes*
	2012	*Yes*	21	*Yes*	*Yes*	*Yes*	*Yes*
	2014	*No*	26	*Yes*	*Yes*	*Yes*	*Yes*
	2014	*Yes*	38	*Yes*	*Yes*	*Yes*	*Yes*
	2015	*No*	54	*Yes*	*Yes*	*Yes*	*Yes*
	2016	*No*	16	*Yes*	*Yes*	*Yes*	*Yes*
	2016	*Yes*	22	*Yes*	*Yes*	*Yes*	*Yes*
L. aspera	2016	*No*	36	*Yes*	*Yes*	*No*	*Yes*

	2016	*Yes*	39	*Yes*	*Yes*	*No*	*Yes*
	2017	*No*	72	*Yes*	*Yes*	*No*	*Yes*
	2018	*No*	38	*Yes*	*Yes*	*Yes*	*Yes*
	2018	*Yes*	38	*Yes*	*Yes*	*Yes*	*Yes*
S. speciosa	2016	*No*	44	*Yes*	*No*	*No*	*Yes*
	2016	*Yes*	37	*Yes*	*No*	*No*	*Yes*
	2017	*No*	71	*Yes*	*No*	*No*	*Yes*
	2018	*No*	38	*Yes*	*Yes*	*Yes*	*Yes*
	2018	*Yes*	41	*Yes*	*Yes*	*Yes*	*Yes*

Table 3 Predictors and associated abbreviation (useful for Tables S1-S5) for models predicting seed set as a function of fire and phenological traits.

Code	Predictor
B	burn term, 0 for no burn, 1 for burn
S	synchrony
B x **S**	interaction between burn and synchrony
U	management unit, only in *E. angustifolia* models
Y	year, only in *E. angustifolia* models
B x **Y**	interaction between burn and year, only in *E. angustifolia* models

Table 4 Summarizing evidence of fire effects on reproductive fitness via resource-dependent and pollination-dependent mechanisms.

Species	Resource-dependent	Pollination-dependent
E. angustifolia and *L. aspera*	Yes, burned plants had higher head count and seeds per plant. Burned plants also had higher fruit counts (due to more heads) but fruits per head did not increase with fire.	Yes, plants that were burned had higher average seed set and variation among individual fitness outcomes (seeds per plant) depended on mating opportunities (flowering synchrony)
S. speciosa	No, fire was not associated with increases in stem, head, fruit, or seed counts.	No, fire did not affect average seed set and variation in outcomes did not track variation in mating opportunities.

APPENDIX 1

Supplement Protocol for cleaning and counting fruits and seeds

Echinacea angustifolia— Heads were harvested when seeds were mature, stored at room temperature for several months, and then dissected to remove all fruits. Fruits

were scanned and then counted from scanned images three times by three different trained volunteers. We germinated, weighed, or x-rayed fruits to assess seed set, or the fraction of fruits that contain a seed. Germination only occurred on samples from 1996-1998 using methods in described in Wagenius 2004 and Wagenius 2006. Starting in 2007, we weighed each fruit in a random sample of 30 fruits from each head using a precision balance using the method described in Ison and Wagenius (2014). Fruits that weighed over 1.95 mg were considered "full". In 2012 and onward, seed set was determined by taking a random sample of 30 fruits and x-raying them on the floor of a Faxitron MX-20 using an x-ray dose of 18kV for 2 s. We then classified these x-ray images, counting the number of seed-containing fruits, empty fruits, and partial fruits in each subsample. The proportion of full + partial fruits to the total number of fruits in the subsample served as our estimate for the plant's seed set. Very few partial fruits were in the samples.

Liatris aspera— At the stage of peak seed maturity, these stalks were harvested and allowed to dry within paper bags. We then dissected each sample, counting the fruits from either one-third of the available full seed heads up to seven heads, or from all heads if one-third of the available full seed heads produced fewer than 30 fruits total. Averaging the fruit counts per head for each plant, we could then multiply the number of heads observed in the field by this average fruit count to estimate the total number of fruits the plant produced during its flowering season. We randomly selected thirty fruits, or all fruits, if fewer than thirty remained on the plant, and placed them in clear plastic bags before x-raying these subsamples with Faxitron MX-20. X-ray dosage was set at 12 kV for 4 s with fruits on the floor of the cabinet. We then classified these x-ray images,

counting the number of seed-containing fruits and empty fruits in each subsample. The proportion of full fruits to the total number of fruits in the subsample served as our estimate for the plant's seed set. One of our three members assigned to *Liatris* head cleaning selected thirty fruits for x-ray analysis from only the heads whose total fruits he had counted. Because the assigned order of specimen cleaning had been randomized, these specimens were randomly dispersed through the data.

Solidago speciosa— One to two stems of each of *Solidago speciosa* plant were randomly selected to be harvested at the stage of peak seed maturity and allowed to dry for several months within a paper bag. We then dissected each sample, preliminarily counting the total number of stems and heads. Next, five heads were chosen at random (though we intentionally disregarded heads which appeared to have lost fruits through wind dispersal or handling), from which we counted present fruits. By averaging the five heads' fruit counts, we determined an approximate value of fruits per head for each individual plant. With this data, we estimated total fruit count for each *Solidago* plant: we multiplied the average fruit count per head by the total number of heads in each sample, then divided this fruit count by the number of stems in the sample to approximate fruits per stem. Thus, this estimation could be multiplied by the number of stalks observed in the field for each plant to determine approximate total fruit count.

Next, we further processed the *Solidago* samples to estimate seed set for each plant. The collection of fruits counted in the previous step (chosen from five randomly selected heads) was supplemented with fruits from an additional three to four randomly selected heads. From this sample of fruits, thirty fruits were chosen at random and placed into a clear plastic bag. We then x-rayed these subsamples using a Faxitron MX-20 x-ray

machine set to 4 s at 12 kV, with samples set on the floor of the cabinet. We analyzed each x-ray image to classify full fruits (those that contained seeds) from empty, aborted, or otherwise damaged fruits. The proportion of full fruits to total number of fruits in the subsample (thirty) reasonably estimates the overall seed set for the plant. During protocol optimization, we tried a few methods thus the first 14 samples were not cleaned in this method; for these, we removed all fruits, spread them onto a grid (either 6x6 or 6x13), and counted fruits from either 3 or 4 grid boxes. The order of cleaning samples was randomized so these samples are randomly dispersed through the data.

FIGURE LEGENDS

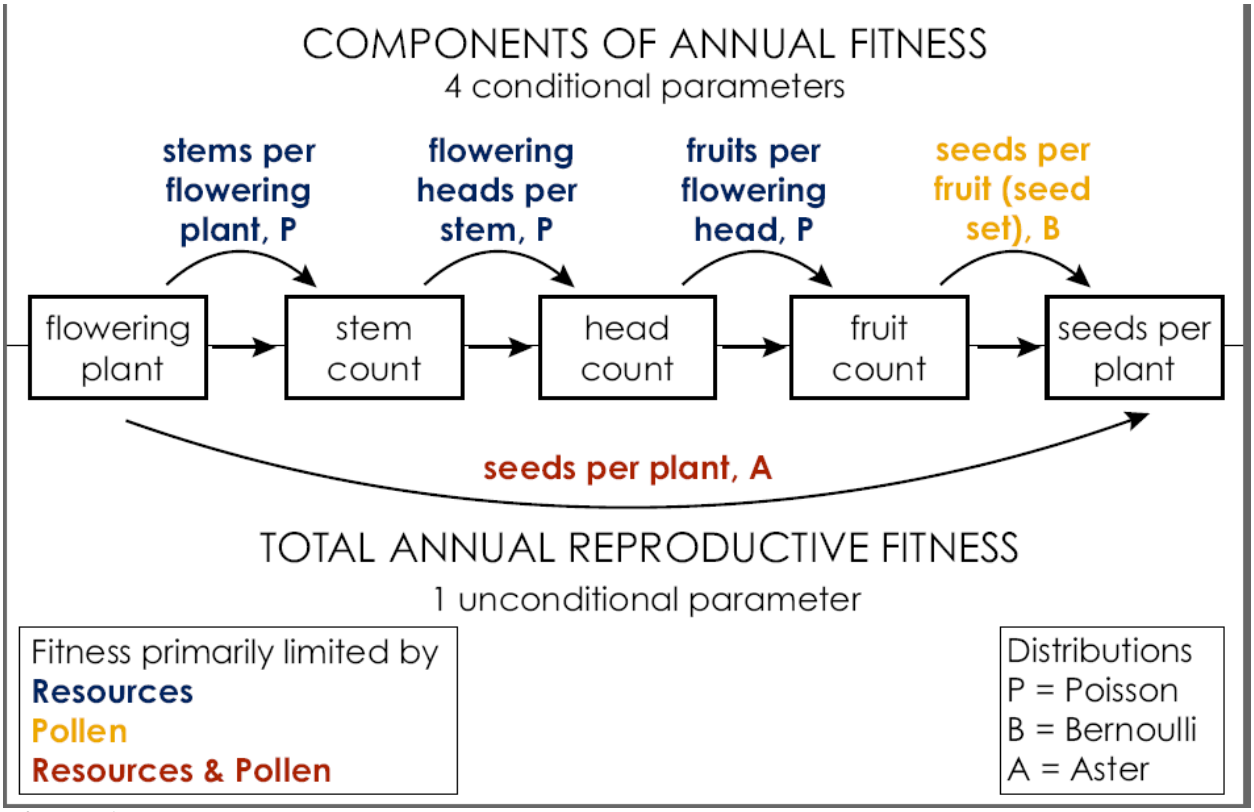

Figure 1 Total seeds per plant, hereafter total reproductive fitness, depends on multiple components. Resource availability and pollination can both affect total reproductive fitness, but they may influence different components of fitness including the counts of flowering stems, heads (capitula), fruits, and seed set. Using aster models, we can

quantify both total reproductive fitness and its components in a single model. We expect that total reproductive fitness may be limited by both resources and pollen, which can both be affected by fire. Therefore, using aster models to understand the relationship between fire, the components of fitness, and total reproductive fitness will advance understanding of the underlying mechanism by which fire affects plant populations in prairies.

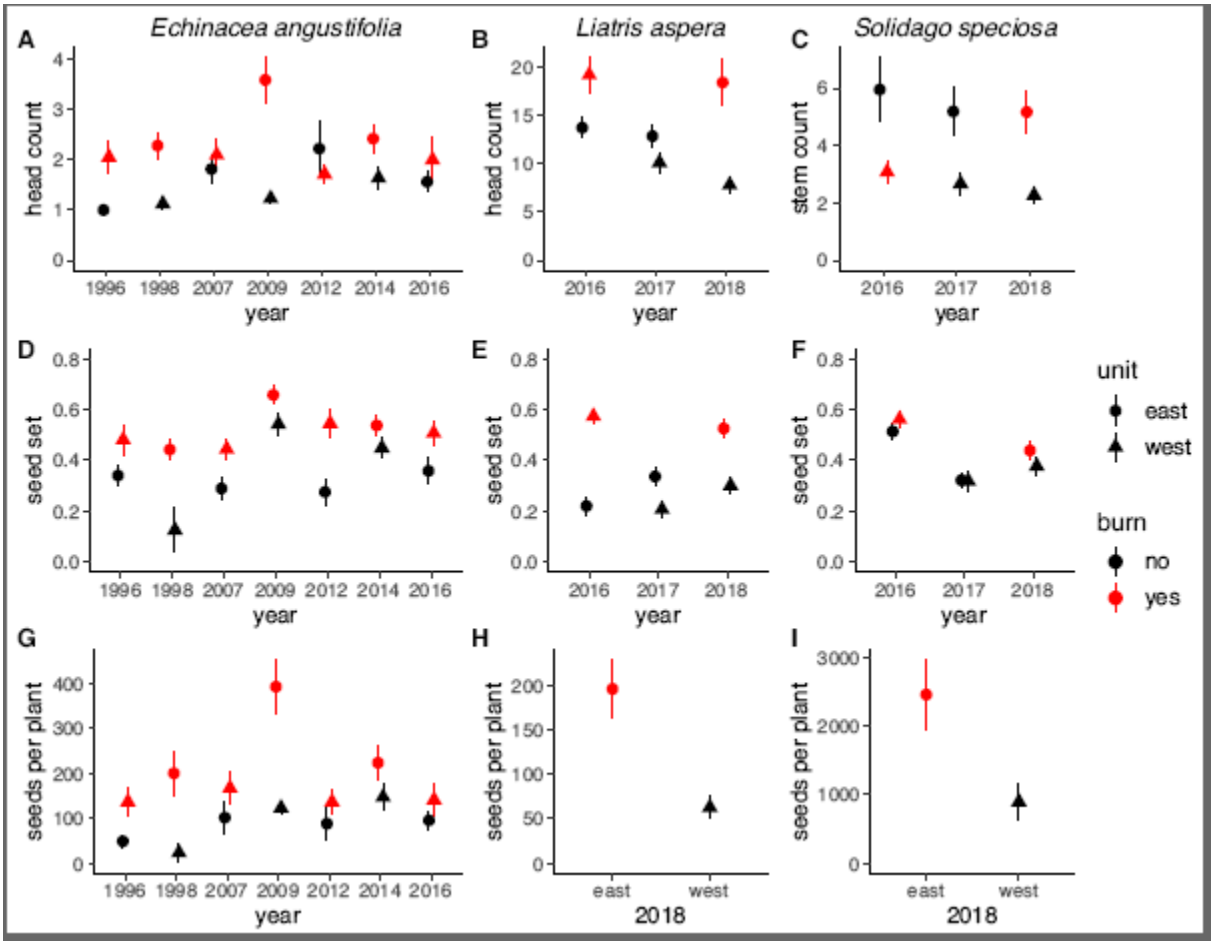

Fig. 2 Means +/- SE of three selected components of reproductive fitness calculated from the complete dataset for *E. angustifolia* (a, d, g), *L. aspera* (b, e, h), and *S. speciosa* (c, f, i). Components of reproductive fitness include counts of flowering stems, heads, fruits, and seeds as defined in Fig. 1. In *L. aspera* and *S. speciosa* we obtained counts of total

seeds in 2018 but not 2016-2018 (as outlined in Table 2 and the methods section). See Table 1 for burn dates in each year.

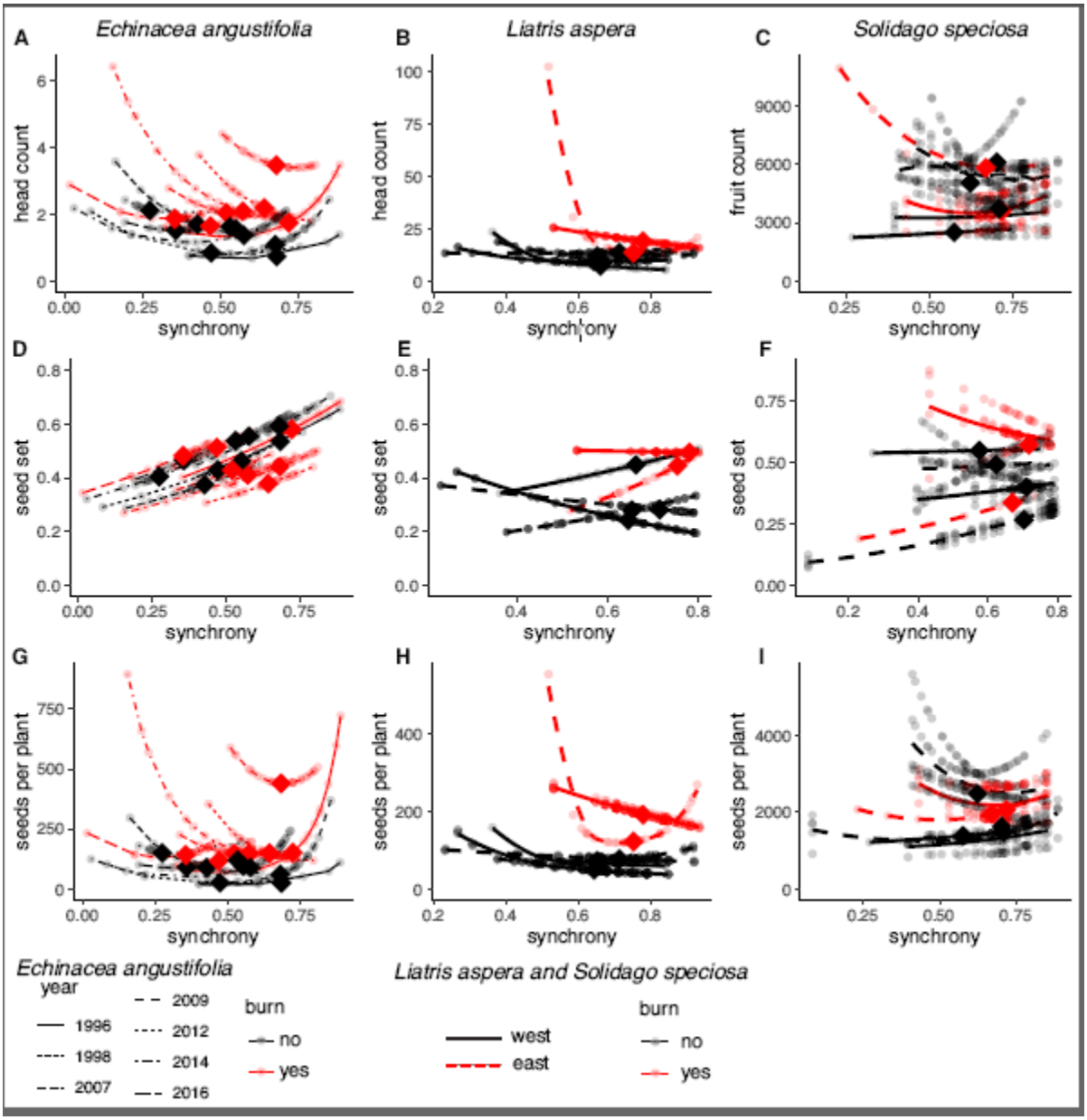

Fig. 3 Fitness components over the range of observed synchrony values for each mating scene, as predicted by the best unconditional aster models for *E. angustifolia, L. aspera*, and *S. speciosa*. The predicted mean of each fitness component at observed mean synchrony per mating scene is denoted with a large, filled diamond, red for burned scenes

and black for unburned scenes. In *L. aspera* and *S. speciosa,* five representative sets of predicted values are shown in the above figure out of the 250 sets of predicted values generated for each mating scene in 2016 and 2017 (see methods for details). Fitness values are predicted over the range of observed synchrony values from each mating scene. The best unconditional aster models in *E. angustifolia* and *L. aspera*, included interaction terms between fire, flowering synchrony, the head count fitness component, and total reproductive fitness (model selection results in Appendix S1, Table S3 and Appendix S1, Table S4). In *S. speciosa* the best unconditional aster model interaction terms between fire, flowering synchrony, the fruit count fitness component, and total reproductive fitness (model selection results in Appendix S1, Table S5).