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Do cone age and heating mode determine the opening of serotinous cones during wildfires? A new bench scale approach applied to *Pinus halepensis* Mill.

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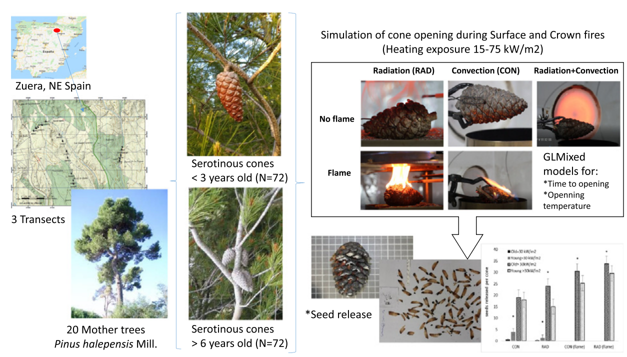
HIGHLIGHTS

- New methodology is a realistic and standardized method for opening serotinous cones.
- Heating mode, cone weight and mother tree have significant effects on cone opening.
- Heating higher than 30 kW m^{-2} generates a significant increase in seed release.
- Seed wings partially affected by fire decreasing the potential dispersion distance
- Experiments confirm the important ecological role of cone age.

GRAPHICAL ABSTRACT



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ABSTRACT

Serotiny is a well-known fire adaptive trait in some species, as the Mediterranean conifer *Pinus halepensis*. However, information about cone opening mechanisms during wildfires and consequences on post fire dispersal is scarce. In addition, standardized methods allowing a realistic simulation of heating modes at bench-scale are not available. In this study, we address for the first time the interacting effects of radiation, convection and direct flame on the opening and seed release of serotinous cones, following a novel repeatable methodology. Using a Mass Loss Calorimeter (MLC) device and a wide range of heat exposures (between 5 and 75 kW m^{-2}) with or without ignition, we intended to simulate realistic cone heating during surface and crown fires in laboratory conditions. Additionally, we included the effect of contrasting serotinous cone ages interacting with heating mode and considering the random individual variation. The proposed methodology has shown a high potential to simulate the complex process of crown fires in relation to cone opening under controlled conditions, detecting a threshold of heat exposure (25–30 kW m^{-2}) for cone opening. We confirmed that heating mode had a highly significant effect in cone opening, interacting with cone age, while cone age effect on its own was marginal. Particularly, ignition significantly increased the efficacy of cone opening and seed release. Moreover, young and old cones behave differently in seed release, both in surface and crown fire simulations. Implementing and adjusting this methodology in other species will allow more realistic and reliable quantitative comparisons than previously attained.

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1. Introduction

Serotiny (the delayed seed dispersal in ripe fruits or cones) is considered one of the key adaptive traits in response to fire in many woody plants, as part of a strategy of 'fire embracing', i.e. the death of adults and successful recruitment from the aerial seed bank (Keeley and Zedler, 1998; Pausas and Keeley, 2014). This strategy includes the presence of a high proportion of dead branches in the crown and a low crown base height, which increase the flammability and hence the probability that crown fires triggered the opening of serotinous cones (Lamont et al., 1991; Pausas, 2017; Resco de Dios, 2020; Schwilk and Ackerly, 2001). Serotiny can be characterized by cone longevity, and the proportion of closed cones in the crown (Lamont et al., 1991). These variables display high variability among pines species and also among provenances or ecotypes within some species (Briand et al., 2015; Ledig et al., 2013; Tapias et al., 2001, 2004).

Pinus halepensis (Aleppo pine) is a typical representative of the fire embracing strategy, with a particularly precocious reproduction and high serotiny, together with high crown flammability and relatively thin bark (Pausas, 2017). Moreover, both ecotypic differentiation and phenotypic plasticity in serotiny degree have been reported (Climent et al., 2008; Martín-Sanz et al., 2016; Santos-Del-Blanco et al., 2013). Some studies have shown morphological and anatomical differences between serotinous and non-serotinous cones of Aleppo pine, being serotinous cones more compact, rigid and consistent, and with scales and seeds providing greater protection against high temperatures reached during fire (Moya et al., 2008; Salvatore et al., 2010). However, other studies have suggested a more continuous, quantitative variation in cone serotiny, with a key role of water supply through the living peduncles to maintain the cones closed (Martín-Sanz et al., 2017). This particular mechanism implies maintenance costs and explains the variable cone opening without fire (xeriscence) that has been repeatedly discussed in relation to Aleppo pine (Espelta et al., 2011; Ne'eman et al., 2004) and is consistent with the plasticity of serotiny –a reduction of plasticity in dry environments – observed in this species (Martín-Sanz et al., 2016).

Although the relationships between cone age, resin content and opening have previously been mentioned (Tapias et al., 2001), no studies have specifically addressed the effect of cone age on cone opening. In general, older serotinous cones have a lower resin content than younger cones but nevertheless maintain the scales closed for a long time (Daskalakou and Thanos, 2004). According to Johnson and Gutsell (1993), serotinous cones remain closed because of resins that seal the cone scales together. If sufficient heat is applied to the cone for a certain period of time, the resin bonds weaken and break, the cone scales bend outwards from the cone axis, and seeds are released. Resin, a defence substance synthesized by plants to protect against pests and diseases, contains high levels of biogenic volatile organic compounds (BVOCs), which are more closely related to the flammability of cones than to protection against fire (Della Rocca et al., 2017; Ormeño et al., 2009).

Although several studies have investigated fire adaptive traits in *P. halepensis*, including serotiny (e.g. Hernández-Serrano et al., 2013; Martín-Sanz et al., 2016, 2017; Ne'eman et al., 2004; Pausas and Keeley, 2014), information about cone opening mechanisms in conditions similar to those observed in wildfires is scarce (Alexander and Cruz, 2012). Moreover, the effect of cone age on cone opening and the potential consequences on post fire seed dispersal are also poorly known (Despain et al., 1996).

Most studies involving cone opening used muffle furnaces (Johnson and Gutsell, 1993; Moya et al., 2008; Salvatore et al., 2010). However, according to some authors, this method does not provide a realistic simulation of the cone heating that occurs in real situations, because the most important heat sources during crown fires are radiation and convection from flames (Cruz et al., 2006). Alexander and Cruz (2012) developed a model for predicting cone opening after crown fires, but highlighted some potential limitations as the model was based on the values obtained by Johnson and Gutsell (1993) in a series of experimental tests using a muffle furnace. Thus, Alexander and Cruz (2012) recommended exploring new methods that take heating by convection into account. Despain et al. (1996) used experimental fires to open serotinous cones, but since they did not calibrate heat exposure, standardization was elusive.

Several protocols have been developed to characterize the flammability of forest fuels, most of which are based on devices designed for building materials, easy to standardize (White and Zipperer, 2010). In previous studies, one such device, the Mass Loss Calorimeter (MLC), was used to characterize dead fuels (Madrigal et al., 2009), live fuels (Madrigal et al., 2013), fuel beds (Della Rocca et al., 2018) and tree bark (Dehane et al., 2015; Madrigal et al., 2019). This device measures the heat release rate (HRR, kW m^{-2}) by means of a chimney that collects combustion gases and a thermopile that is calibrated by methane gas. One important advantage of this apparatus is that it enables different heating configurations for comparing cone opening by convection, radiation (Espinosa et al., 2020; Madrigal et al., 2019) or a combination of both in a reproducible method. In addition, the device allows the presence or absence of a pilot flame or spark that promotes controlled ignition of the cones.

The aims of this study were (1) to develop a new methodology to characterize the opening of serotinous cones during simulated fires at bench-scale and (2) to assess the effect of different heating modes (convection, radiation, presence of flame) on the opening and seed release of serotinous cones with different age. We hypothesized that (i) heat exposure level and heating mode (radiation, convection) during fire will cause differences in cone opening and potential dispersal after fire and (ii) the age of serotinous cones will affect the opening process during wildfires and their post-fire seed dispersal.

2. Material and methods

2.1. Study area and sampling

Sampling was conducted in a natural, even-aged stand of *Pinus halepensis* Mill. located in Zuera (province of Zaragoza, North-Eastern Spain), where studies on the genetics and plasticity of this species have previously been carried out. We chose 20 trees at random along 3 transects selected in tracks avoiding border effect to represent the aerial seed bank of studied stand. Trees were even-aged (20 years old), with similar characteristics (total height = 6.24 ± 1.29 m, dbh = 17.3 ± 4.1 cm, crown base height = 1.92 ± 0.76 cm, bark thickness = 2.9 ± 0.5 cm) (coordinates X = 672000 Y = 4641000, datum ETRS89, UTM H30) (Fig. 1).

The climate in the study area is Mediterranean with a continental influence (average temperature 16 °C, annual precipitation 541 mm), characterized by a wide temperature range between summer and winter, with a maximum temperature of 41.9 °C and a minimum temperature of −9.6 °C. Estimated fire rotation period based on the Spanish Forest Administration database ranges between 50 and 250 years, with more than 1000 ha burned in the period 1974–2005 (Vázquez de la Cueva et al., 2015).

We separated two different groups of serotinous cones per individual based on the estimated cone age (Tapias et al., 2001). Therefore, we considered young cones, if we were sure that they were less than 3 years old, and old cones if they were more than 6 years old before the sampling (maximum estimated cone age was 12 years old). We randomly selected cones within each group (72 cones), hence totalling a sample of 144 cones. We then measured each cone (length, width and weight) before the opening test.

2.2. Experimental device and test protocols

We used an adapted Mass Loss Calorimeter (FTT®) (Madrigal et al., 2009, 2013, 2019) to simulate cone opening under different heating

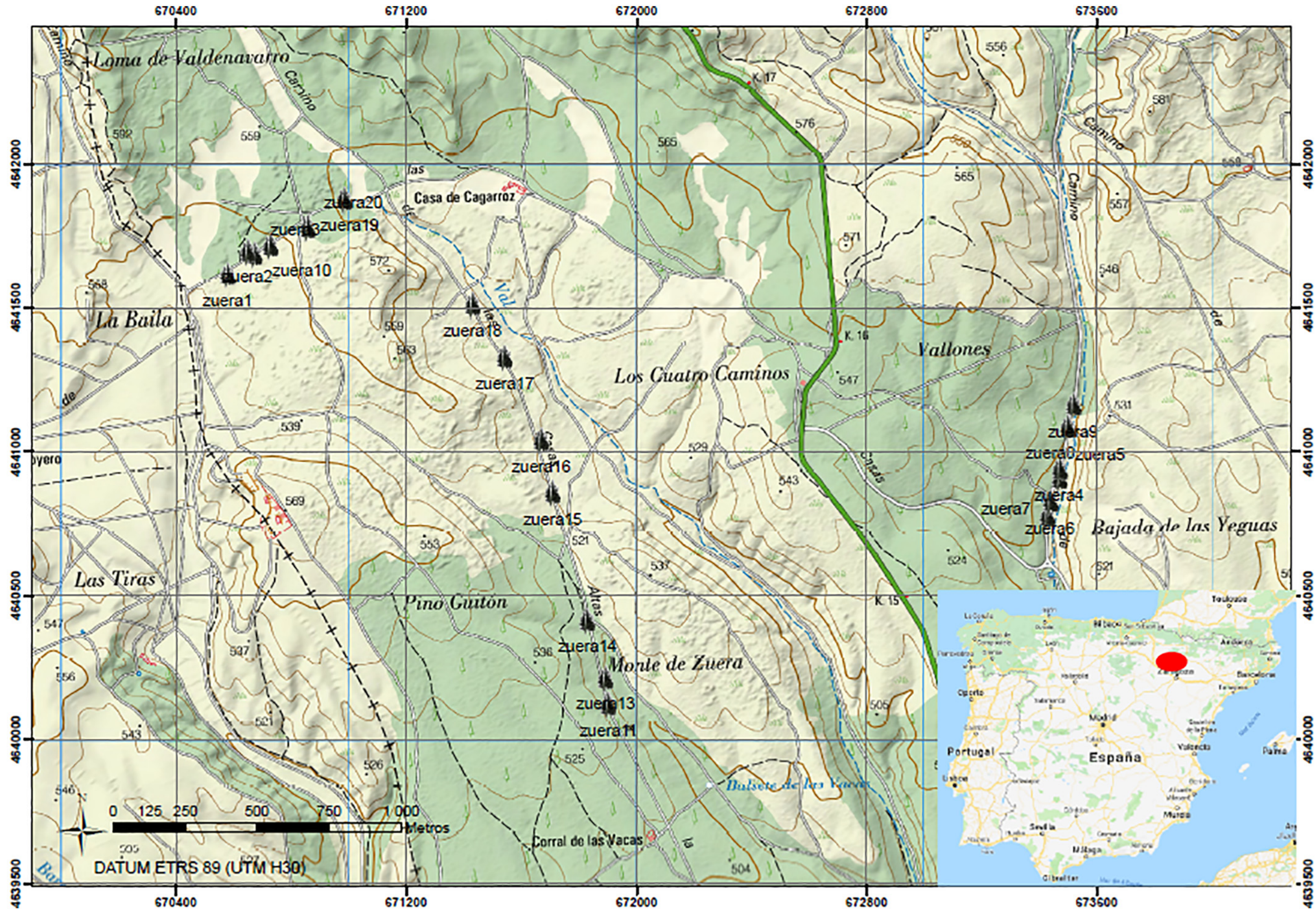

Fig. 1. Location of study area and selected transects. Sampled trees in each transect are shown.

treatments: radiation, convection and radiation + convection. As mentioned above, the device is designed to expose a sample to a radiant heat flux and to record the test reaction (heat release rate) using a chimney and a calibrated thermopile to measure the temperature of gases (enthalpy method: for more details, see Madrigal et al., 2011). The thermopile is calibrated by different fluxes of methane gas. In this study, we propose exposing serotinous cones to different heat convection fluxes to simulate the effect of convection column during wildfire or prescribed burning. Different convective fluxes were achieved by adjusting methane flow rate (Madrigal et al., 2009). The procedure was completed using a calibrated epiradiator to expose samples simultaneously to convection and radiation heat (see below). During wildfires, ignition sources are frequently present in the convection column (e.g. embers, sparks). During crown exposure to a surface fire in which the heat released is higher than 15–40 kW m^{-2} (Cruz et al., 2006), the high temperatures (higher than 600 K) and the presence of ignition sources in contact with flammable substances can lead to the ignition of canopy fuels. We simulated such scenario with a piloted ignition by means of a spark generator for two radiant heat fluxes (25 and 50 kW m^{-2}) and using a pilot flame for the highest convective heat flux (40 kW m^{-2}).

In summary, the following series of tests were conducted, with at least three replicates per treatment, to comply with repeatability criteria (Madrigal et al., 2009). A total of 144 tests were completed (Table 1, Fig. 2):

1) Treatment 1: Tests exposing cones to radiation using a calibrated radiant heater. We applied calibrated radiant heat fluxes of 5, 15, 25, 35 and 50 kW m^{-2}, and series were carried out for non-piloted ignition, simulating the effect of heat transfer by radiation in the crown.
2) Treatment 2: Tests combining heat fluxes of 25 kW m^{-2} and 50 kW m^{-2} and piloted ignition (generating a sparkle using an electrode) in order to force ignition of the cone. The sparkle generator

Table 1
Summary of the different protocols to induce cone opening at bench-scale for different heating mode, heat exposure and the presence or absence of piloted ignition for two ages of serotinous *Pinus halepensis* cones (young <3 years old; old >6 years old). Total sample N = 144.

Heating mode	Treatment code	Heat exposure (kW m^{-2})	Ignition (yes/no)	Young cones (N)	Old cones (N)
Radiation	1	5	No	6	6
Radiation	1	15	No	6	6
Radiation	1	25	No	6	6
Radiation	1	35	No	6	6
Radiation	1	50	No	6	6
Convection	3	10	No	3	3
Convection	3	15	No	3	3
Convection	3	25	No	3	3
Convection	3	30	No	3	3
Convection	3	40	No	3	3
Radiation + convection	5	45	No	3	3
Radiation + convection	5	50	No	3	3
Radiation + convection	5	60	No	3	3
Radiation + convection	5	65	No	3	3
Radiation + convection	5	75	No	3	3
Convection	4	40	Yes	6	6
Radiation	2	25	Yes	3	3
Radiation	2	50	Yes	3	3
Total (N)		–	–	72	72

Fig. 2. (a) Experimental device (b–f) different protocols used to open serotinous cones (g) characterization of cones before and after test and seed release. (a) Mass Loss Calorimeter (MLC) device showing (1) calibrated radiant heater used to produce different radiant heat fluxes (2) data logger for recording temperatures in cones with type K thermocouples (3) chimney calibrated to produce different levels of convective heating (4) digital video system for recording tests (5) MLC control unit. Examples of the tested heating modes are shown: (b) cone opening test with radiation (c) cone opening test with convection (d) cone opening test with convection + radiation (e) cone opening test with convection and piloted ignition (f) cone opening test with radiation and piloted ignition. Sparkle generator was sited at 5 mm over the cone (g) example of cone before and after test showing the seed release one week after test.

was placed in the plume of flammable gases at 5 mm over the cone (see Fig. 2f)

3) Treatment 3: Tests exposing cones to convection of a calibrated plume of methane gas. According to the measurement with calibrated heat flux transducers (Medtherm®) to estimate convective heat flux (see below), the methane gas fluxes corresponded to 10, 15, 25, 30, 40 kW m^{-2} of convective heat. Series of tests were carried out for non-piloted ignition, simulating the effect of a convection column in the crown.
4) Treatment 4: Tests using Treatment 3 configuration with a heat flux of 40 kW m^{-2} but adding piloted ignition.
5) Treatment 5: Tests exposing cones to combined radiation (calibrated epiradiator using a heat flux of 35 kW m^{-2}) and convection (see point 3), yielding a total heat exposure of 45, 50, 60, 65, 75 kW m^{-2}.

We recorded the tests with a digital video to determine both the time to ignition and the time to cone opening. We used a type K thermocouple (1 mm diameter) connected to an automatic data logger with 1 s frequency (1 Hz) to monitor temperatures under scales during tests without flame (Fig. 2).

The convective heating for Treatment 3, 4 and 5 was developed by fixed fluxes of methane gas according to the specifications of MLC device. The proposed "custom made configuration" measured the heat transfer (kW m^{-2}) emitted by methane gas using calibrated heat flux transducers (Medtherm ®). Radiant and convective heat fluxes were in the same order of magnitude but they had not the same value and range. Calibrated heat transfer (kW m^{-2}) using total heating (radiative and convective) measured in this way was added (Treatment 5).

The cones were exposed to different heat fluxes during 60 s, simulating an average flame resident time and rate of spread under natural conditions (Alexander and Cruz, 2012).

After the tests, the cones were weighed and then placed in aluminium trays under room conditions (20 °C, 35% relative humidity) for one week before the number of seeds released was counted as a proxy for potential seed dispersal after a fire, assuming that part of them (unknown) are trapped into the cone. We carried out an additional calculation of relative seed release as the proportion of seed released compared to the total number of seeds per cone.

2.3. Experimental design and data analysis

Generalized Linear Mixed Models (GLMM) were used to detect the potential influence of fixed factors: cone age (two levels: young and old cones) and heating mode (included as dummy variables: radiation, convection, presence of flame according to Table 1). The following response variables were selected: time to opening (time elapsed between the start of heating treatment and the opening of the first scale), opening temperature (temperature under the scale at the time to opening), mass loss (difference in weight before and after the test, expressed as a percentage), number of released seeds and relative seed release (released seeds one week after test). The distribution of predicted variables was checked to ensure compliance with parametric test requisites (skewness, kurtosis, influence points, homogeneity of variances) and for selecting the suitable link function (Gaussian, Gamma or Negative binomial distribution). Total heat exposure (kW m^{-2}) and cone weight (g) were included in the model as continuous predictor covariates.

Once the set of potential explanatory covariates was selected and the hierarchical nature of the data (multiple cones in a single tree) taken into account, a multilevel linear mixed model including both fixed and random effects acting at tree level (mother tree) was fitted. Interactions between fixed factors (age of cone and heating mode) were also analysed.

After the model was fitted, the assumptions of normality and homoscedasticity and potential autocorrelation of the residuals were evaluated. Finally, the goodness of fit statistics, referring to both the marginal (not considering random effects) and conditional (including random effects) predictions, were determined. The best model was selected using the corrected Akaike criterion value (AICc).

All data analyses were conducted using SPSS® software.

3. Results

The mean values of response variables for different cone age and heating treatment (Appendix Table A.1) showed that the greatest differences were due to heating treatment. Time to opening ranged between 3 and 11 s among treatments but the average value for young and old cones was similar (8–9 s). Opening temperatures ranged between 48 and 49 °C for radiation + convection (Treatment 5), 57–59 °C for radiation (Treatment 1) and 81–93 °C for convection (Treatment 3). The overall mean for both young and old cones was between 62 and 67 °C. Cone mass loss showed no difference between cone ages (averaging 4%) but a significant treatment effect, with ignition treatments (2 and 4) showing higher mass loss (8–13%) compared to treatments without ignition (1, 3 and 5, 1–3%). Overall mean of released seeds after treatments was 10 seeds per cone. Nevertheless, we detected evident differences related to ignition treatments (2 and 4). In these cases, the number of seed released was higher than 17 seeds per cone, while in Treatments 1, 3 and 5 (without ignition) the value was below 10 seed per cone. The percentage of seeds released per cone was between 12 and 48% for young cones and 7–53% for old cones. Results showed a high correlation between total seed release and percentage of seed release per cone ($r = 0.841$, $p < 0.01$). The GLMM (Table 2) revealed significant effects of the considered factors and covariates on the response

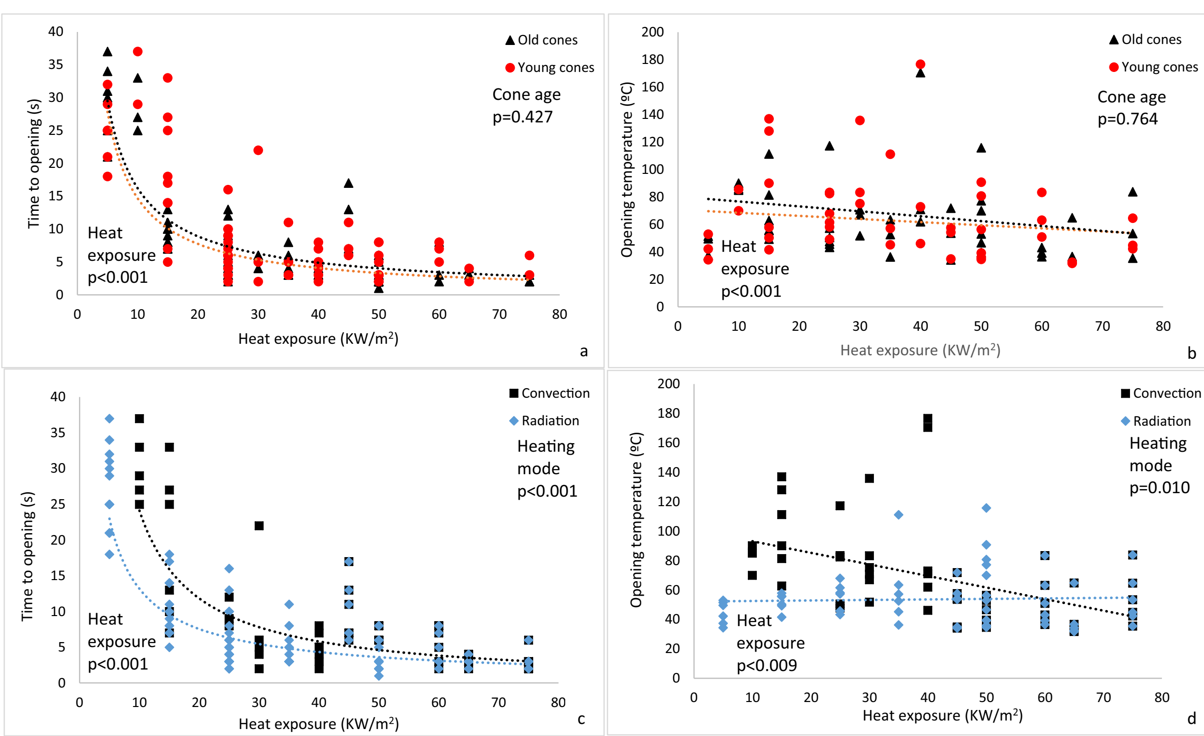

Fig. 3. Representation of all series of tests according to their heat exposure and cone age. All points are classified as convection or radiation heat flux (including ignition treatments) and the overlapped points in (c) and (d) represent radiation + convection tests. (a) Non-linear correlation ($y = 118.65x^{-0.864}$; $R^2 = 0.55$) between "Time to opening" and "Heat exposure" for cone age (young and old cones) (b) linear correlation ($y = -0.36x + 80.23$; $R^2 = 0.051$) between "Opening temperature" and "Heat exposure" for cone age (young and old cones) (c) non-linear correlation between "Time to opening" and "Heat expo sure" for heating mode (convection $y = 256.20x^{-1.03}$; $R^2 = 0.57$ and radiation $y = 83.76x^{-0.80}$; $R^2 = 0.58$) (d) linear correlation between "Opening temperature" and "Heat exposure" for heating mode (convection $y = -0.79x + 100.98$; $R^2 = 0.24$ and radiation $y = 0.035x + 52.16$; $R^2 = 0.002$).

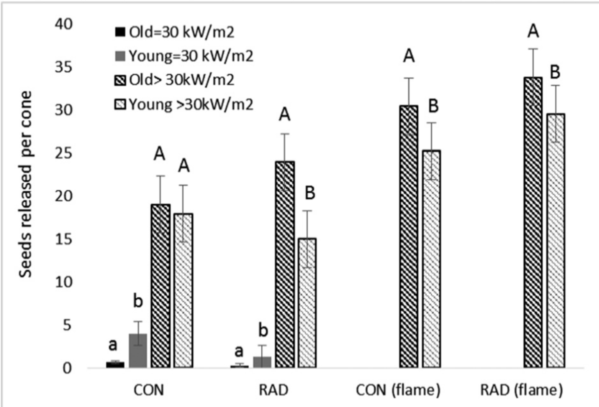

Fig. 4. Interactions between heating mode (convection, radiation and flame) and cone age (Young < 3 years and Old > 6 years old of cones) for different simulated heating modes (see Table 1) and the number of seeds released per cone. Different letters show significant differences ($p < 0.05$) between young and old cones for each heat exposure (30 kW m^{-2} lower letters and higher than 30 kW m^{-2}capital letters). Seeds were not released at heat exposure lower than 30 kW m^{-2}. CON = convection, RAD = radiation, CON (flame) = convection + flame, RAD (flame) = radiation + flame.

variables. The model confirmed the significant effects of heating mode and heat exposure for all variables. Cone age alone was not a significant factor for predicting time to opening, opening temperature and mass loss, but it had a significant effect on seed release such that older cone released more seeds, and interactions with the heating mode were significant. The presence of flame (Treatments 2 and 4) produced a significantly higher seed release. Cone weight had a marginally significant negative effect ($p < 0.1$) on the time to opening and opening temperature, but a high and positive significant effect ($p < 0.001$) on seed released. In addition, the random individual tree effect was significant for all response variables ($p < 0.05$). Plot of time to opening versus heat exposure (Fig. 3a) for cone age (young vs. old) showed an exponential trend with a fast opening above 25–30 kW m^{-2}. This trend was not detected for opening temperature (Fig. 3b). The exponential trend for predicting time to opening differed significantly in relation to the heating mode (convection vs. radiation) (Fig. 3c). Opening temperature was constant for different radiation exposure, but the decreasing linear trend was significant for convection heating mode (Fig. 3d).

The GLMM model showed significant interactions between cone age and heating treatment for the number of seeds released per cone (Table 2). Seed release below 30 kW m^{-2} was marginal and mostly occurring in young cones. By contrast, above 30 kW m^{-2} (with a more abundant cone opening and seed dispersal) older cones released significantly more seeds than young ones both for radiation and ignition treatments (Fig. 4). This trend is similar for relative number of seeds released per cone in % (Appendix Table A.1).

4. Discussion

In this study, we address for the first time the effects of radiation and ignition on the opening of *Pinus halepensis* serotinous cones in a laboratory bench experiment.

The results confirmed that heating treatment significantly affected all variables analysed. We detected an exponential relationship between heat exposure and time to cone opening. An exponential, yet less steep relationship (i.e. lower value of exponent) between temperature and time to opening was described by Johnson and Gutsell (1993) in studies conducted in an open muffle oven with pilot flame for *Pinus banksiana* Lamb. and *Pinus contorta* var. *latifolia* Engelm cones. The results suggest that our method generates a more realistic thermal shock, reducing the time to break the cone scale seals (Alexander and Cruz, 2012). Studies based on heating models implemented in a muffle oven (therefore only with radiation heating) showed that ignition of *Pinus banksiana* and *P. contorta* cones occurred at temperatures above 600 °C (Johnson and Gutsell, 1993). This temperature belongs to a radiative heat transfer of approximately 50 kW m^{-2} according to our experiment. By contrast, we demonstrated that cone ignition is possible at lower heat fluxes (25 kW m^{-2}) if a spark was applied. Thus, the presence of flame strongly modifies the opening mechanism of scales, and therefore results obtained in muffle ovens can be less representative of a crown fire. These findings are consistent with the idea that the presence of flame and convection heat strongly modify cone opening (Alexander and Cruz, 2012). To our understanding, this is the first time that this mechanism has been demonstrated using a standardized methodology (Despain et al., 1996).

We found that cones opened quickly (<10 s) when heat exposure exceeded 25–30 kW m^{-2}. According to the physical model proposed by Cruz et al. (2006), crown fires (at least passive crown fires) will be initiated at 15–20 kW m^{-2} of radiative heat and 10–20 kW m^{-2} of convective heat (potential total heat between 10 and 40 kW m^{-2}). Therefore, the experimentally obtained values are rather close to those observed in a real crown fire. The mean temperature under the scales required for opening cone scales was 64 °C (although it varied between 32 and 118 °C), a temperature slightly higher than the opening temperature observed for this species in tests conducted in warm water (50.7 °C, Tapias et al., 2001) and in a muffle oven (50 °C, Martín-Sanz et al., 2017). However, in tests carried out in a dry-air stove, Habrouk

Table 2
Generalized Linear Mixed Models (GLMM) for response variables. The coefficient (Coeff) and p values are shown for each predictor. Factor variables are binary (dummy variable), with Coeff shown for one level. Heat exposure and Cone weight were included in the models as continuous covariates. The significance values for random effect of the tree variable are shown. The distribution function of response variables, selected link function and corrected Akaike criterion values (AICc) for each GLMM model are shown. Significance values higher than 95% ($p < 0.05$) are highlighted in bold.

	Time to opening		Opening temperature		Mass lost		Dispersed seed	
	Coeff	p	Coeff	p	Coeff	p	Coeff	p
Intercept	4.786	**<0.0001**	3.772	**<0.0001**	−2.386	**0.035**	−25.823	**0.029**
Cone = Young	−0.228	0.427	−0.036	0.764	−1.357	0.340	−36,234	**<0.0001**
Radiation = 0	−1.144	**<0.0001**	0.750	**<0.0001**	1.794	**0.015**	6.171	**0.037**
Convection = 0	−1.361	**<0.0001**	0.373	**0.010**	2.086	**0.002**	13.130	**0.002**
Flame = 0	0,110	0.490	nc	nc	0.344	**0.005**	−23.498	**0.006**
Heat exposure (kW m^{-2})	−0.051	**<0.0001**	0.007	**0.009**	0.078	**<0.0001**	0.668	**<0.0001**
Cone weight (g)	−0.009	0.079	−0.009	0.054	−0.016	0.345	1.023	**<0.0001**
ConeAge × RAD	0.362	0.120	0.148	0.362	0.907	0.514	33.109	**<0.0001**
ConeAge × CON	0.141	0.525	0.037	0.821	0.869	0.523	12.559	**0.003**
ConeAge × FLAME	0.212	0.327	nc	nc	0.577	0.967	21.206	**0.018**
Random (Tree)	–	**0.025**	–	**<0.0001**	–	**<0.0001**	–	**<0.0001**
Distribution function	Negative binomial		Negative binomial		Negative binomial		Gauss Inverse	
Link function	Logistic		Logistic		Logistic		Identity	
AICc	229.386		30.86		138.49		635.67	

et al. (1999) observed that *P. halepensis* cones did not open below 70 °C. Therefore, temperatures of 60–65 °C can be considered the average threshold at which resins will break and the scales will thus open, although the value will vary greatly in cones from the same and different species (Johnson and Gutsell, 1993). Even when we found significant differences with only 3–5 replicates per treatment, number of replications should be higher in future experiments to deal with larger variability among cones.

Cone opening temperature was approximately constant as previously cited (Briand et al., 2015; Johnson and Gutsell, 1993; Ledig et al., 2013; Tapias et al., 2001). By contrast, for convection exposure, cone opening temperature differed significantly after exposure to low and high heat. These results suggest that resins sealing the scales will be more resistant to low heat of convection (surface fire), increasing time to opening (Fig. 3b) and requiring higher temperatures under the scales (Fig. 3d). The contrasting effect of serotinous cone opening for surface and crown fires has previously been described, showing that most of these cones only open during wildfires, known as *pyrescence* (e.g. Keeley et al., 2011; Lamont et al., 1991; Pausas and Keeley, 2014). Our findings indicate that the key differences are the heating mode (convection) and the detected threshold of heat exposure (25–30 kW m^{-2}) for crown fire initiation (Cruz et al., 2006).

Against our expectations, the age of the cones did not affect significantly the time to opening under our experimental conditions. Previous studies in Aleppo pine, with a different heating protocol and different water supply through the living peduncles, showed a significantly faster opening (less time or lower temperatures) in older cones (Martín-Sanz et al., 2017). Other authors also suggested that cone aging is an important variable affecting cone opening during fires (Salvatore et al., 2010). However, the cone aging effect is likely to be more related to the gradual loss of xeriscence in Aleppo pine (significantly affected by water supply, Espelta et al., 2011; Martín-Sanz et al., 2017), which is probably overridden by the major effect of intense heating mimicking a crown fire. Nevertheless, our findings show that old serotinous cones do not behave in the same way as young cones, particularly in seed release.

Cone weight had a slightly significant effect on time to opening and opening temperature and a highly significant effect for the number of released seeds (Table 2): the higher the cone weight, the lower the time to opening and opening temperature and the more seeds released. These results confirm the important effect of cone weight in controlling biological fire traits (Calvo et al., 2016).

When heat exposure is below 30 kW m^{-2} (surface fire or limit of crown fire initiation, according to Cruz et al., 2006), cone opening is less effective (Fig. 3). This is logical in physical terms, because before the arrival of a fire front to the crown, convection heating is the main process that transports energy (Cruz et al., 2006) and the cones do not generally burn (corresponding to the simulated conditions of convection without flame). Our findings showed that the lower heating at which at least one seed is released is 30 kW m^{-2}, with higher number of seed released in young cones than in old cones. However, heat fluxes greater than 30 kW m^{-2} generated a significant greater seed release in old cones than in young cones (Fig. 4). These findings confirm the important ecological role of cone age: young cones are the main portion of aerial seed bank that will disperse seeds during surface fires and old cones will mainly disperse seeds during crown fires (Daskalakou and Thanos, 2004; Ne'eman et al., 2004). In this respect, the precocious opening of old xeriscent cones due to heatwaves and/or intense droughts, as postulated in previous studies (Espelta et al., 2011; Martín-Sanz et al., 2017), may be highly detrimental for recruitment after crown fires.

Noteworthy, the seeds did not burn during the tests, confirming the efficient insulation of cone scales (Moya et al., 2008). Under these conditions and with time to exposure lower than 60 s, it is reasonable to expect that the loss of seed viability will be low or null (Habrouk et al., 1999). However, after seed release, all seed wings were partially burned in the presence of flame during tests (Fig. 2g), therefore decreasing the potential dispersion distance relative to that of seed released from unaffected cones (Nathan et al., 1999). These findings have biological sense and are consistent with the detected interactions (Table 2, Fig. 4) because after a high intensity crown fire (ignition of cones) the adult trees are usually killed and dispersion of seeds by wind will be less critical than after surface fires. After surface fire, the presence of wind-dispersed seed will be important to regenerate in gaps when most of the stand survived (Rigolot, 2004). Additional experiments at stand scale are needed to confirm these results. Additional questions about the effects of type of heating in the viability of seeds and potential interactions between seed weight, dispersion and heat must be clarified.

The proposed methodology has shown a high potential to simulate the complex process of crown fires in relation to cone opening under controlled conditions, thereby complying with most of the suggestions and observations made by Alexander and Cruz (2012) about the need of developing a more realistic method for opening serotinous cones. The findings open up new research lines exploring the use of this methodology with other species or with adapted benches or intermediate scale devices (White and Zipperer, 2010).

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CRediT authorship contribution statement

J. Madrigal: Conceptualization, Methodology, Formal analysis, Writing – original draft, Funding acquisition. **A. Martín:** Investigation, Data curation, Formal analysis. **R. Chambel:** Investigation, Resources. **M. Guijarro:** Writing – review & editing. **C. Hernando:** Writing – review & editing. **M. Callejas:** Visualization. **J. Espinosa:** Visualization, Writing – review & editing, Supervision. **J. Climent:** Supervision, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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