Immunity priming uncouples the growth-defense tradeoff in tomato

Running title: Uncoupling growth-defense tradeoffs

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Summary statement

Growth-defense tradeoffs in plants result in loss of yield. Here, we demonstrate that immunity priming in different pathways uncouples this tradeoff and allows for disease resistant plants with robust growth.

Abstract

Plants have developed an array of mechanisms to protect themselves against pathogen invasion. The deployment of defense mechanisms is imperative for plant survival, but can come at the expense of plant growth, leading to the "growth-defense trade-off" phenomenon. Following pathogen exposure, plants can develop resistance to further attack. This is known as induced resistance, or priming. Here, we investigated the growth-defense trade-off, examining how defense priming via Systemic Acquired Resistance (SAR), or Induced Systemic Resistance (ISR), affects tomato development and growth. We found that defense priming can promote, rather than inhibit, plant development, and that defense priming and growth tradeoffs can be uncoupled. Cytokinin response was activated during induced resistance, and found to be required for the observed growth and disease resistance resulting from ISR activation. ISR was found to have a stronger effect on plant development than SAR. Our results suggest that growth promotion and induced resistance can be co-dependent, and that in certain cases, defense priming can drive developmental processes and promote plant yield.

45 **Introduction**

46

47 Plants often face abiotic and biotic stresses such as pathogen infection or climate/
48 ecological changes. These stresses induce physiological, biochemical, and molecular
49 changes, which can be reflected in altered development, lowered growth, and
50 reduced productivity. Plants lack mobile immune cells and an adaptive immune
51 system, and protect themselves against pathogens using several complex
52 mechanisms. In the interaction between the plant and the attacker, elicitor
53 molecules of various types are released (Mishra et al., 2012). Perception of elicitors
54 by the host plant results in signaling that leads to activation of various defense
55 mechanisms (Walters and Heil, 2007).

56

57 Following pathogen infection, plants can develop enhanced resistance to further
58 attack. This is known as induced resistance, and is broadly divided into systemic
59 acquired resistance (SAR) and induced systemic resistance (ISR) (Walters and Heil,
60 2007). SAR, commonly triggered by local infection, can provide long-term resistance
61 to subsequent infection (Klessig et al., 2018). During SAR, pathogenesis-related (PR)
62 genes are activateds. Among the best-characterized PR genes is *PR-1*, which is often
63 used as a marker for SAR (Pieterse et al., 2014). SAR generally relies on salicylic acid
64 (SA), which has been shown to increase in plant tissues during pathogenesis
65 (Yalpani et al., 1991) . The SA receptor NPR1 (Non-expressor of Pathogenesis
66 Related genes 1) regulates SAR signaling following SA perception. NPR1 is activated
67 by SA, and subsequently activates transcription of genes required for SAR, including
68 PR genes (Cao et al., 1997; Chen et al., 2021; Pieterse et al., 2014).

69

70 Unlike SAR, which is usually activated during pathogen infection, induced systemic
71 resistance- ISR is usually described as being initiated by beneficial soilborne
72 microorganisms, including rhizobacteria and fungi. ISR is associated with a
73 physiological state in which plants can react more efficiently to pathogen attack
74 (Shoresh et al., 2005), known as immunity "priming". ISR is regulated by jasmonic
75 acid (JA) and ethylene (ET), and is typically not associated with a direct activation of
76 PR genes, although *NPR1* can enhance ISR and, in some cases, *PR-1* is accumulated

77 during ISR priming (Gupta and Bar, 2020; Meller Harel et al., 2014; Pieterse et al.,
78 1998). As a result of priming, upon secondary infection, defense responses occur
79 more rapidly and strongly than during the primary infection, enabling a more
80 effective response to the new infection (Heil and Bostock, 2002).
81
82 While the deployment of defense mechanisms is imperative for plant survival,
83 defense activation can come at the expense of plant growth. This is known as the
84 "growth-defense tradeoff" phenomenon, and is the result of a balance between
85 growth and defense/ adaptation responses (Figueroa-Macías et al., 2021). Growth–
86 defense tradeoffs have an important role in plant survival (Huot et al., 2014). In
87 general, mature organs and tissues are no longer growing and, as a result, can be
88 more prepared for defense as they possess more energy and resources that can be
89 mobilized. One recent work suggested that competition for resources could shape
90 the costs of defense induction (Karasov et al., 2017). Growth-defense trade-offs in
91 plants have been increasingly investigated of late (Ke et al., 2020; Margalha et al.,
92 2019; Monson et al., 2022; Saini and Nandi, 2022). Although some works have
93 suggested that growth and defense are antagonistic, the extent of the
94 "development-defense-tradeoff" and the mechanisms by which it affects plant
95 growth and agricultural yield are largely uninvestigated.
96
97 Phytohormones involved in both developmental processes and defense, could
98 potentially be involved in regulating development-defense tradeoffs. The
99 developmental phytohormone Cytokinin (CK) is involved in cell division, leaf
100 senescence, apical dominance, vascular differentiation, root development and
101 stress responses (Zürcher and Müller, 2016). CKs have reported roles in response to
102 biotic stresses (Großkinsky et al., 2011; Gupta et al., 2020; Jiang et al., 2013), and
103 promote resistance through the SA signaling pathway (Choi et al., 2010; Gupta et
104 al., 2020). We have previously shown that CK-deficiency results in higher disease
105 susceptibility, while high endogenous CK content, as well as external application of
106 CK, confer disease resistance (Gupta et al., 2020). Gibberellins (GA) are plant growth
107 hormones involved in stem elongation, germination, leaf expansion, flowering, and
108 fruit development (Davière and Achard, 2013). GAs regulate growth by destabilizing

109 DELLA, a negative regulator of GA signaling (Locascio et al., 2013). GAs can
110 negatively regulate JA signaling, potentially affecting disease resistance (Hou et al.,
111 2010). The central roles of CK and GA in plant growth and development, together
112 with their reported connections with biotic stresses, suggest that these two
113 hormones could be involved in growth- or development-defense tradeoffs.

114

115 SA and JA, which were classically defined as "defense/ stress" hormones, also
116 regulate developmental processes (van Butselaar and Van den Ackerveken, 2020;
117 Ghorbel et al., 2021; Rivas-San Vicente and Plasencia, 2011; Wasternack et al.,
118 2013). This suggests that growth and defense may not be simply antagonistic, but
119 rather, have a more complex balance (Koo et al., 2020). Rather than looking at
120 growth and defense as a binary switch, a more holistic view suggests that plant
121 fitness is optimized when growth and defense are appropriately prioritized in
122 response to both environmental and developmental cues. Researching the
123 molecular mechanisms used by plants to balance growth and defense can lead to
124 the possibility of manipulating these mechanisms, with the goal of increasing plant
125 productivity.

126

127 In this work, we aimed to investigate the growth-defense trade-off in tomato, by
128 examining how defense priming affects tomato development and agricultural
129 qualities. We used three different immunity elicitors, ASM (Acibenzolar-S-Methyl),
130 also known as BTH (benzo (1,2,3) thiadiazole-7-carboxylic acid) and marketed under
131 the trade name "bion" (Oostendorp et al., 2001), a SAR elicitor, *Trichoderma*
132 *harzianum*, a classical fungal ISR elicitor (Elad, 2000a), and *Bacillus megaterium*, a
133 bacterial elicitor which likely induces both SAR and ISR (Gupta et al., 2021a, 2022a).
134 These elicitors were employed to investigate differences and commonalities
135 between SAR and ISR pathways, and examine their effect on tomato development.
136 We found that both SAR and ISR affect plant development, with ISR generating a
137 more pronounced positive effect on agricultural qualities. We show that growth and
138 defense can be positively correlated, as mutants that lost the ability to activate
139 defenses, also lost the growth effects. In addition, we found that cytokinin response

140 is activated in response to ISR, and is required for the observed growth and disease
141 resistance resulting from ISR activation. Taken together, our results suggest that
142 growth promotion and induced resistance can be co-dependent, and that defense
143 priming can, within a certain developmental window, not necessitate a trade-off on
144 growth, but rather, encourage and drive developmental processes.
145

146 **Results**

147 *Activation of SAR and ISR both promote resistance to B. cinerea and increase plant*
148 *immunity*

149 The elicitors *Trichoderma harzianum* T39, a *Trichoderma* sp. fungus that induces
150 immunity via the JA/ET pathway (Meller Harel et al., 2014), ASM (Acibenzolar-S-
151 Methyl), a SA analog that primes the SA pathway (Huot et al., 2014), and *B.*
152 *megaterium* "4C", a phyllosphere *Bacillus* isolate that promotes plant development
153 and immunity and likely activates both SAR and ISR pathways (Gupta et al., 2021a,
154 2022a), were used to compare SAR and ISR induction of pathogen resistance.
155 Elicitors were applied via foliar spray. Further details on immunity priming
156 methodology are provided in the methods section. We conducted *Botrytis cinerea*
157 infection assays on 4-5-week-old primed *S. lycopersicum* M82 plants. We found that
158 pre-treatment with all three elicitors reduced disease levels (**Fig. 1**). Foliar spray or
159 soil-drench application of all three elicitors had a similar effect on disease resistance
160 (**Fig. S1**).

161 Induction of plant immunity was also examined using both SAR and ISR elicitors. We
162 measured ethylene and ROS (reactive oxygen species) production in 4-5-week-old
163 plants. These assays are indices of immunity activation (Leibman-Markus et al.,
164 2017). We found that pre-treatment via foliar spray with the ISR elicitor T39, the
165 SAR elicitor ASM, or the combined SAR/ISR elicitor 4C, all amplified wounding
166 ethylene production and ethylene production mediated by the fungal MAMP
167 (microbe associated molecular pattern) EIX (ethylene inducing xylanase, see (Anand
168 et al., 2021)) in *S. lycopersicum* M82, as compared to mock-treated plants (**Fig. 1C-**

169 **D**). Pre-treatment with T39, ASM, or 4C, also amplified ROS production in response
170 to the bacterial PAMP (pathogen associated molecular pattern) flg22 (**Fig. 1E**).

171 *Activation of SAR or ISR differentially promote plant development and productivity*

172 To examine possible growth-defense tradeoffs, we treated plants separately with
173 elicitors, inducing immunity via ISR with T39, via SAR with ASM, or via both
174 pathways, with 4C. ASM concentrations were calibrated in preliminary assays (**Fig.**
175 **S2**; see methods section for further details). Plants were soil-drenched with the
176 different elicitors once a week for 4 weeks, and grown to harvest, and agricultural
177 parameters were sampled. All three elicitors promoted plant growth (**Fig. 1F**),
178 however, only T39 and 4C were able to significantly promote yield and Harvest
179 index (**Fig. 1G-H**). ASM significantly reduced yield. Total tomato soluble sugars, as
180 measured by Brix refractometry, were unaffected (**Fig. 1I**), suggesting that immunity
181 elicitation did not affect metabolic processes governing fruit quality.

182 We next examined whether immunity elicitation could affect seedling development
183 (**Fig. 2**). 10-day-old seedlings were spray-treated with elicitors once and harvested
184 after a week for growth and development quantification, or given two treatments
185 one week apart, and harvested one week after the second treatment. All three
186 elicitors affected seedling height (**Fig. 2A, B**), though ASM was only able to do so
187 after two treatments (**Fig. 2B**), while T39 and 4C increased plant height after one
188 treatment (**Fig. 2A**). T39 and 4C also increased seedling fresh weight (**Fig. 2C, D**) and
189 the number of leaves produced by the seedling (**Fig. 2E, F**). ASM did not affect fresh
190 weight or leaf numbers. Representative images of treated seedlings (**Fig. 2 G-J**),
191 meristem and four youngest leaf primordia (**Fig. 2 K-N**), and fifth leaves (**Fig. 2 O-R**)
192 are provided. A more in depth analysis of the effect of T39 on development
193 demonstrated that T39 treatment resulted in increases in the height of the shoot
194 apical meristem (SAM) but not the cotyledons (**Fig. S3A,E,F**), indicating that the
195 treatment affected hypocotyl growth mostly above the SAM. T39 treatment also
196 promoted the number of unfurled leaves (**S3B,G**), the developmental plastochron
197 (**Fig. S3C, H**) and the meristematic differentiation to flowering (**Fig. S3D, I**). We
198 observed that T39 promotes leaf development, increasing the number of leaves
199 produced (**Fig. S4A**), and leaf complexity (**Fig. S4B-D**) (**Fig. S4A, B**). T39 did not alter

200 the developmental program of leaves 1-2, which are simple and have a short
201 developmental window in tomato (Shleizer-Burko et al., 2011) (**Fig. S4C**). We
202 previously reported that 4C treatment promotes the leaf developmental program,
203 increasing leaf complexity and patterning (Gupta et al., 2022a). ASM had no effect
204 on leaf development (**Figs. 2, S4**).

205

206 *The balance between cytokinin and gibberellin is involved in altered development in*
207 *response to immunity elicitation*

208 We next examined possible routes through which T39, ASM, and 4C promote
209 development. The balance between CK and GA determines the leaf developmental
210 window in tomato (Israeli et al., 2021). As we observed that the leaf developmental
211 window was extended upon T39 and 4C treatment, with additional organs being
212 produced (**Figs. 2, S3-S4,** see also (Gupta et al., 2022a)), we hypothesized that
213 changes in CK and/or GA content or response might be involved. Therefore, we
214 assayed CK and GA pathway gene expression and content, in plant leaves spray-
215 treated with the different elicitors, in both source (**Fig. 3**) and sink (**Fig. S5**) leaves.
216 Additional details are provided in the methods section. In source leaves, CK
217 biosynthesis (*IPT*- isopentenyl transferase genes (Žižková et al., 2015)), activation
218 (*LOG*- lonely guy genes (Naseem et al., 2015)), and degradation (*CKX*- cytokinin
219 dehydrogenase genes (Cueno et al., 2012)) were up-regulated by all elicitors (**Fig.**
220 **3A-C**), with some differences. CK degradation appeared more strongly activated by
221 ASM and 4C as compared with T39 (**Fig. 3C**). T39 and 4C treatment resulted in an
222 increase of isopentenyl (iP) in source leaf tissue (**Fig. 3G**), and 4C treatment
223 increased isopentenyl riboside (iPR) content. Despite gene expression results, ASM
224 treatment did not increase CK content, and resulted in reduction in the content of
225 transzeatin (tZ, **Fig. 3G**). Indeed, CK signaling (*TRR*- Tomato Response regulator
226 genes (Bar et al., 2016)) was promoted by T39 and 4C, but not ASM (**Fig. 3D**). *TRRs*
227 are numbered based on their homology to *Arabidopsis ARR*s, such that, for
228 example, *TRR3/4* is homologous to both *ARR3* and *ARR4* of *Arabidopsis*, and so on.
229 GA biosynthesis genes (*GA20 oxidase* and *GA3 oxidase* (Shohat et al., 2021)) were
230 both up and down regulated upon ASM or 4C treatment, while T39 caused a small

231 up-regulation in *GA3ox2* (**Fig. 3E**). The GA degrading *GA2ox7* (Shohat et al., 2021)
232 was up regulated following T39 and ASM treatment, but not 4C treatment (**Fig. 3F**).
233 All three elicitors caused an increase in GA4 and a decrease in GA7 (**Fig. 3G**).
234 Changes in the expression levels of both *GA20ox* and *GA3ox* can explain the shift
235 between these two GA compounds (Hedden, 2020). To confirm defense pathway
236 activation, we assayed the expression of the classical SAR gene *PR1a* (Silverman et
237 al., 2005) and the classical ISR gene *LoxD* (Mariutto et al., 2011), and the ratio
238 between the expression of these two genes was calculated (**Fig. 3H**). While we
239 cannot rule out activation of both pathways, a ratio below one is indicative of ISR
240 being the primary defense pathway, while a ratio above one suggests that SAR is
241 activated. Our results suggest SAR as the primary pathway for ASM, as previously
242 reported (Huot et al., 2014), and ISR as the primary pathway for T39, as previously
243 reported (Meller Harel et al., 2014). The interim, above one value for 4C supports
244 combined ISR and SAR activation with this bacterium, as we previously suggested
245 (Gupta et al., 2021a, 2022a).

246 In sink leaves, CK and GA were much harder to detect, likely due to the smaller
247 amount of available tissue. However, alterations to CK and GA pathway genes were
248 also observed in sink leaves (**Fig. S5**). Treatment with 4C activated one *IPT* and one
249 *LOG* gene (**Fig. S5A-B**). All treatments promoted CK degradation (**Fig. S5C**). T39
250 increased the expression of three *TRR* genes, 4C of two *TRR* genes, and ASM- of one
251 *TRR* gene (**Fig. S5D**). GA biosynthesis was activated by 4C, and repressed by T39
252 (**Fig. S5E**), and GA degradation was repressed by both T39 and ASM (**Fig. S5F**). CKs
253 and GAs were mostly undetectable in our sink tissues. Transzeatin was unchanged
254 by elicitor treatments, and 4C treatment effected an increase in GA7 (**Fig. S5I**), in
255 accordance with the parallel increase in *GA20ox1* (**Fig. S5E**). Developmental
256 transcription factors, which were not detected in the source leaves, were up-
257 regulated in sink leaves (**Fig. S5G**). The meristem maintenance KNOX gene *TKN2*
258 (Hake and Ori, 2002) and the organ boundary determination gene *GOBLET- GOB*
259 (Berger et al., 2009), were upregulated upon treatment with T39 and 4C (**Fig. S5G**).
260 KNOXI proteins can negatively affect GA levels by repressing the GA biosynthesis
261 gene GA20ox and/or activating the GA inactivation gene GA2ox. Thus, TKN2 could

262 increase the CK/GA ratio by negatively regulating GA levels, which was shown to
263 mediate the function of KNOXI in modulating compound leaf development (Bolduc
264 and Hake, 2009; Hay et al., 2002; Jasinski et al., 2005; Sakamoto et al., 2001). ASM
265 treatment caused up-regulation of *GOB* but not *TKN2*. *Clausa* (*CLAU*), a
266 differentiation promoting transcription factor (Bar et al., 2016), which is known to
267 reduce CK sensitivity and increase GA sensitivity (Bar et al., 2016; Israeli et al.,
268 2021), was unaffected (**Fig. S5G**). The ratio between the expression of *PR1a* and
269 *LoxD* (**Fig. S5H**) was similar to that observed in source leaves (**Fig. 3H**).

270 The balance between CK and GA, rather that content of each hormone, was
271 reported to be important in leaf development (Fleishon et al., 2011; Israeli et al.,
272 2021). Since we observed increases in CK content and signaling upon elicitor
273 treatments (**Fig.s 3, S5**), we further examined CK signaling in response to elicitors
274 using tomato plants expressing the synthetic cytokinin response promoter *pTCSv2*
275 (two component sensor) driving VENUS fluorescent protein expression (Steiner et
276 al., 2020). Ten-day-old *pTCSv2*-VENUS expressing seedlings received one elicitor
277 treatment, and were imaged after 48 h. All three elicitors increased the CK-response
278 mean fluorescent signal in the meristem and youngest leaf primordia (m+3), while
279 T39 alone increased the mean fluorescent signal in older leaf primordia (P4-P5) (**Fig.
280 4A-B**). Corrected total fluorescence (CTF), a measure of the total amount of CK-
281 response signal taking into account the fluorescent area and background readings,
282 was unaffected by ASM , but increased in response to T39 and 4C treatment, in
283 meristems and youngest primordia (m+3), as well as in older primordia (P4-P5) (**Fig.
284 4C**). Thus, T39 and 4C increased both the local signal intensity within the meristem
285 and three youngest leaf primordia, and the total signal measured in all tissues, while
286 ASM increased the local signal intensity in the meristem and three youngest leaf
287 primordia, but did not affect the total CK-response signal (**Fig. 4A-C**; See also (Gupta
288 et al., 2022a)). This correlates with the effect of these elicitors on TRR gene
289 expression in sink tissues (**Fig. S5**), as expected (Steiner et al., 2020; Zürcher et al.,
290 2013).

291

292 Since these results indicated that CK, or the ratio between CK and GA, is required to
293 transduce the developmental signal generated by immunity induction with elicitors,
294 we examined the response to T39, ASM, and 4C treatments, in several altered
295 hormone content and/or signaling lines. Genotypes with high CK content or
296 signaling are generally disease resistant, while genotypes with low CK content or
297 signaling can be disease susceptible (Gupta et al., 2020). Since the ratio between CK
298 and GA can determine developmental outcomes (Israeli et al., 2021), we included
299 both high and low CK, and high and low GA genotypes in the study.

300 We found that the high CK or low GA lines behaved similar to the M82 background
301 line in growth, development, and *B. cinerea* resistance upon treatment with all
302 three elicitors (**Fig.s 5A-B, C-D, G-H, K-L**, and **S6A-B, C-D, G-H, K-L**). However, T39
303 and 4C were no longer able to promote growth (**Fig. 5E, I**), development (**Fig. S6E-F,**
304 **I-J**), or *B. cinerea* resistance (**Fig. 5 F, J)** upon reduced CK (**Fig. 5E, F**) or increased GA
305 signaling (**Fig. 5I, J**). ASM promoted disease resistance irrespective of CK or GA
306 signaling (**Fig. 5B,D,F,H,J,L**), and continued to promote growth upon reduced CK
307 signaling (**Fig. 5E**), but did not affect leaf development in any of the lines (**Figure**
308 **S6A-L**). Some differences were observed in the level of response of the different
309 lines to the different elicitors (**Fig. S7**), with responses to T39 and 4C significantly
310 lower in *IPT* and *clau* than in M82. Thus, T39 and 4C, which induce ISR, increase CK
311 biosynthesis and response (**Fig.s 3-4**), and cannot promote growth and
312 development in the absence of a functioning CK pathway (**Fig.s 5, S6**). ASM, which
313 induces SAR, does not promote CK biosynthesis (**Fig.s 3**), has minor effects on CK
314 response (**Fig. 4**), does not require a functioning CK pathway to promote growth
315 (**Fig. 5**), and does not promote leaf development (**Fig. S6**). Though 4C induces both
316 SAR and ISR, the ISR effects are dominant in the context of growth and
317 development, and are promoted irrespective of SAR induction.

318

319 *Blocking SAR or ISR in tomato mutants leads to loss of disease resistance*

320 To further examine the relationship between development and defense, we used
321 defense mutants. Tomato mutants compromised in jasmonate and ethylene

322 pathways have been previously shown not to respond to *Trichoderma* with disease
323 reduction in certain cases (Jogaiah et al., 2017; Korolev et al., 2008), while lines
324 compromised in the salicylic acid pathway are expected not to respond to ASM
325 (Oostendorp et al., 2001). Thus, we used the SA-lacking transgene *NahG* and its
326 background line Moneymaker (Mm) (Brading et al., 2000), the reduced JA mutant
327 *spr-2* and its background line Castelmart (Cm) (Li et al., 2003), and the reduced
328 ethylene sensitivity mutant *neveripe* (*nr*) and its background line Pearson (Pn)
329 (Lanahan et al., 1994), and examined their disease response to elicitation with T39,
330 ASM, and 4C. The mutant lines responded as expected to *B. cinerea* infection: *spr-2*
331 with increased susceptibility (AbuQamar et al., 2008), and *nr* and *NahG* with similar
332 susceptibility (Mehari et al., 2015; Audenaert et al., 2002). All the background lines
333 responded to the three elicitors with reduction in *B. cinerea* incited disease (**Fig. 6**),
334 as was observed for M82 (**Fig. 1**). However, *NahG* did not respond to ASM or 4C
335 (**Fig. 6A**), while *spr-2* and *nr* did not respond to T39 or 4C (**Fig. 6B-C**).

336

337 *Blocking SAR or ISR in tomato results in loss of developmental promotion and yield*
338 *increases*

339 Given that mutating defense pathways prevented disease reduction by elicitor pre-
340 treatment, we examined whether the developmental effects of these elicitors
341 would also be abolished in the same mutants. Plants of the different mutant
342 genotypes and their background lines were soil-drenched with elicitors once a week
343 for 4 weeks. All background lines responded to the elicitors with increases in height
344 (**Figure 6D-F**). Once again, *NahG* did not respond to ASM or 4C (**Fig. 6D**), while *spr-2*
345 and *nr* did not respond to T39 or 4C (**Fig. 6E-F**).

346 We further examined the ability of the elicitor treatments to promote side shoot
347 production and leaf complexity, parameters that are known to be CK-dependent
348 (Mller and Leyser, 2011; Shani et al., 2010; Werner et al., 2001). ASM did not affect
349 either of these parameters in WT lines, but was included as a control in case it
350 might have effects on the mutant lines. T39 and 4C increased side shoot production
351 and leaf complexity in all background lines (**Fig. S8**). While *NahG* responded

352 similarly to its background line Moneymaker in response to T39, it did not respond
353 to 4C (**Fig. S8A, D**). *spr-2* and *nr* did not respond to T39 or 4C (**Fig. S8B-C, E-F**). While
354 *spr-2* had significantly higher side-shoot production than the Cm background line
355 even without elicitor treatment, not all buds were activated, with many plants
356 having only 2-4 activated side-shoots (**Fig. S8B**), therefore, the lack of activity of T39
357 and 4C on side shoot activation in *spr-2* is not likely to be due to system saturation.
358 ASM had no effect on side shoot or leaflet generation in either WT lines or defense
359 mutants (**Fig. S8**). T39 and 4C promoted yield in all the background lines, but not in
360 *spr-2* or *nr* (**Fig. S9A-C**). T39 was able to promote yield in *NahG,* but 4C was not (**Fig.**
361 **S9A**). Untreated *NahG* and its background Mm, and *nr* and its background Pn, had
362 similar disease resistance and developmental parameters. *spr-2* baseline disease
363 resistance was lower than its background Cm, and some of the *spr-2* developmental
364 parameters were also altered (**Fig.s 6, S8**).

365 We also examined seedling development in defense mutant genotypes (**Fig.s 7,**
366 **S10**). Ten-day-old seedlings were spray-treated with elicitors twice, one week apart,
367 and harvested on day 24, one week after the second treatment. All elicitors
368 promoted seedling height in the background lines (**Fig. 7A-C**). As observed with M82
369 (**Fig.s 2, S6**), T39 and 4C increased the number of leaves produced (**Fig. S10A-C)**, as
370 well as leaf complexity (**Fig. S10D-F**). As observed with the mature plants (**Fig.s 6,**
371 **S8-S9**), *NahG* did not respond to ASM or 4C (**Fig. 7A)**, while *spr-2* and *nr* responded
372 to ASM (**Fig. 7B-C**), but did not respond to T39 or 4C (**Fig.s 7B-C, S10B-C, E-F**). In
373 seedlings, untreated *NahG* and its background Mm had similar leaf development,
374 while *nr* and *spr-2* had altered leaf development when compared with their
375 respective background lines Pn and Cm (**Fig. S10**). Untreated seedlings of all mutant
376 lines had similar height as their background lines (**Fig. 7**).

377 Thus, the competence to respond to each elicitor in increased immunity, is the
378 same as the competence to respond in altered development and increased growth
379 and yield.

380

381 *Defense and developmental pathways may have overlapping components*

382 Cellular defense responses are activated by T39 (**Fig. 1**; Gupta et al., 2022b; Gupta
383 and Bar, 2020), ASM (**Fig. 1**; Marash et al., 2022), and 4C (**Fig. 1**; Gupta et al.,
384 2021a). ASM treated plants responded to EIX (a JA pathway molecular inducer)
385 more strongly than 4C treated plants, while T39 treated plants responded to flg22
386 (a SA pathway molecular inducer) more strongly than ASM or 4C treated plants (**Fig.**
387 **1**). This could indicate crosstalk or cross-potentiation between SAR and ISR, which
388 was previously suggested (Vlot et al., 2021). To further investigate the relationship
389 between development and defense, we examined whether there could be a genetic
390 overlap between these processes. In previous work (Israeli et al., 2021) we
391 generated a dataset of genes that promote morphogenesis. Our approach, which
392 was validated, relied on published data (Ichihashi et al., 2014) that includes
393 transcriptomics of *S. lycopersicum* cv M82 at four developmental stages. Here, to
394 examine whether we could identify an overlap between morphogenesis and
395 defense pathways, we re-analyzed the previously published data set using KEGG
396 (Kyoto Encyclopedia of Genes and Genomes), to identify additional significantly
397 differential pathways over-represented in morphogenesis. The "plant-pathogen
398 interaction" pathway was found to be significantly enriched in the morphogenetic
399 dataset (Fisher's exact test, $p<0.05$). Genes of the plant-pathogen interaction
400 pathway found to be significantly enriched in the morphogenetic group are
401 provided in **Supplemental Table 1**. Defense functions that were found to be
402 upregulated include pattern recognition receptors, mitogen activated protein
403 kinases, WRKY transcription factors, membrane localized NADPH oxidases, calcium-
404 dependent protein kinases, cyclic nucleotide-gated channels, calcium sensors, and
405 nitric oxide synthase. Most of the cellular immune response pathway is represented
406 in the morphogenesis group, suggesting possible genetic overlap between
407 development and defense (see **Supplemental Table1** and **Fig. S11**).

408

409 **Discussion**

410 *Activating plant defense does not always generate a growth tradeoff*

411 The concept of growth-defense tradeoff is based on the notion that the limited
412 resources available to an organism must be prioritized according to internal and
413 ambient conditions the organism experiences. In plants, which are sedentary and
414 lack circulatory immunity, the role of ambient conditions, including biotic stresses,
415 becomes more prominent, because plants have limited ability to regulate their
416 environment. Early research of growth defense tradeoffs was partially prompted by
417 observations that plants with highly active defense pathways or defense hormone
418 imbalances can be highly resistant to pathogens, but have severely impaired growth
419 (Huot et al., 2014; Vos et al., 2013). However, many of these reports investigated
420 severely pleiotropic mutants, and dramatic changes to hormonal pathways and
421 hormone levels can have severe impacts on development, particularly considering
422 that defense hormones also have developmental roles (De Vleesschauwer et al.,
423 2014; Vos et al., 2015; Wasternack et al., 2013). Growth-defense tradeoffs have
424 been suggested to rely on crosstalk between different hormones. Cross-talk
425 between CK (Albrecht and Argueso, 2017; Cortleven et al., 2019) or GA (Huot et al.,
426 2014; Monson et al., 2022; Yu et al., 2022) , and JA, SA, or ET, has been previously
427 suggested to affect the balance between defense pathways and growth
428 development.

429 Constitutively active plant defense can result in growth inhibition (Gómez-Gómez et
430 al., 1999; Zipfel et al., 2006), although genetically increasing plant immunity was
431 reported to lack negative effects on plant growth or development in several cases
432 (Cao et al., 1998; Heidel et al., 2004; Leibman-Markus et al., 2021; Pizarro et al.,
433 2020). This suggests that the mode of defense activation, whether by external or
434 genetic means, can affect the level of the "tradeoff", opening the possibility for
435 activating defense without significantly impairing plant development, a highly
436 sought after agricultural advantage.

437 *Defense priming can evade negative tradeoffs and result in growth promotion*

438 Previous reports (reviewed in (Gupta and Bar, 2020)) have shown that immunity
439 priming agents can affect plant growth. The underlying hypothesis of the present
440 work was that certain priming agents can in fact improve plant growth, breaking the
441 growth defense tradeoff. To examine this, we selected three known defense

442 priming agents: the SA analog ASM, also known as BTH, a molecular priming agent
443 known to activate SAR (Ishii et al., 2019), the fungus *T. harzianum* T39, a biological
444 priming agent known to activate ISR (Elad, 2000a), and the phyllosphere bacterium
445 *B. megaterium* "4C", which we previously demonstrated likely activates both SAR
446 and ISR (Gupta et al., 2022a, 2021a).
447

448 Our work demonstrates that defense priming can result in both induced resistance
449 and plant growth and development promotion (**Figs. 1-2, 5-7**). T39, ASM, and 4C, all
450 induced resistance against *B. cinerea*. We selected *B. cinerea* as a model pathogen,
451 though this was also reported for all three inducers with additional pathogens
452 (Gupta and Bar 2020; Gupta et al., 2021a; Marolleau et al., 2017;). ASM promoted
453 vertical growth, while T39 and 4C promoted several developmental parameters in
454 both seedlings and mature plants, including agricultural traits (**Fig.s 2,5,S3,S4,S6,S8-**
455 **S10**). Of note is that treatments with *T. harzianum T39* were previously found to
456 activate resistance to downy mildew in grapevine, without negative effects on plant
457 growth (Palmieri et al., 2012). We previously reported that *B. megaterium* 4C
458 promotes both disease resistance (Gupta et al., 2021a) and plant development and
459 growth, including yield (Gupta et al., 2022a). Though ASM did not positively impact
460 most growth parameters when applied to tomato at 0.001%, it also did not have a
461 negative effect on them in the applied concentration, which could be significant
462 agriculturally under a high disease burden. Higher concentrations of ASM were
463 found to inhibit growth (**Fig. S2**).
464

465 *Roles for CK and GA in growth-defense tradeoffs*

466 As central growth hormones, it is perhaps expected that CK and GA should be
467 involved in regulating growth-defense tradeoffs. In recent years, CK has been
468 established as a priming agent, and its role as a defense hormone is increasingly
469 recognized (Choi et al., 2010; Gupta et al., 2020). In the context of disease
470 resistance, most reports indicate that CK promotes immunity and disease resistance
471 through the SA pathway (Choi et al., 2010; Gupta et al., 2020), though roles in JA-
472 mediated defense cannot be ruled out. CK and GA have both previously been

473 suggested to be involved in growth-defense tradeoffs (Cortleven et al., 2019; Yang
474 et al., 2012). CK signaling through response regulators was shown to be regulated
475 following pathogen infection, in several systems (Argueso et al., 2012; Gupta et al.,
476 2020; Igari et al., 2008). CK was suggested to either promote (Choi et al., 2010) – or
477 inhibit (Argueso et al., 2012; Choi et al., 2010) - growth and defense simultaneously,
478 rather than effecting a tradeoff, although experimental evidence of this has thus far
479 been limited.
480 Notably, JAZ–DELLA interactions integrate JA and GA signaling, such that elevated
481 GA levels or response enhance JAZ repression of defense, while elevated JA levels
482 enhance DELLA-mediated repression of growth (Howe et al., 2018; Yang et al.,
483 2012). Thus, under high GA signaling, plants are more resistant to pathogens that
484 require the SA pathway (Navarro et al., 2008). In many cases, the ratio between CK
485 and GA is the determining factor of the developmental (Israeli et al., 2021) or
486 disease (**Fig. 5**) outcome.
487
488 Here, we observed that priming immunity and growth with T39, 4C, or ASM also
489 induced CK signaling (**Figs. 3-4, S5**). CK response was induced more strongly by T39
490 and 4C than by ASM (**Fig. 4B-C, Fig. 3D, Fig S5D**). CK production was induced by T39
491 and 4C, but not ASM (**Fig. 3G**). GA biosynthesis and degradation were affected by all
492 elicitors (**Fig. 3**). The promotion of CK signaling by elicitors is unlikely to be
493 explained merely by the changes we observed in hormone levels, and is no doubt a
494 result of increases in CK sensitivity following elicitor application. Notably, the *clausa*
495 mutant possesses significantly reduced levels of many CKs (bar 2016), and increased
496 levels of pre-active GAs (Israeli et al., 2021), but displays increased sensitivity to CK,
497 reduced sensitivity to GA, high-CK developmental phenotypes, and increased
498 disease resistance (Bar et al., 2016; Israeli et al., 2021), demonstrating that
499 hormone sensitivity, and not hormone quantity, determine growth, development,
500 and disease resistance. Thus, the alterations we observed in gene expression
501 following elicitor treatment, can either serve to promote increases in hormone
502 sensitivity, or be the result of feedback relationships aimed at controlling the level
503 of such increases, as previously reported (Müller, 2011; Sun, 2008). Interestingly,
504 ASM promoted plant height and induced disease resistance under CK attenuation,

505 and continued to support disease resistance upon increased GA signaling, while T39
506 and 4C were not able to promote increases in plant height or disease resistance
507 under CK attenuation or increased GA signaling (**Fig. 5**). Leaf development was
508 promoted by T39 and 4C, but not ASM (**Fig.s 2, S3, S4, S6**). Increased numbers of
509 initiated leaves and increased leaf complexity that resulted from T39 or 4C
510 treatment, could no longer be promoted in low CK or increased GA genotypes (**Fig.**
511 **S6**). Thus, T39 and 4C treatment can uncouple growth-defense tradeoffs, in a
512 manner dependent on the CK-GA balance. Interestingly, though CK requires SA to
513 induce immunity and promote disease resistance (Choi et al., 2010; Gupta et al.,
514 2020), ASM did not appear to require CK in order to induce immunity and promote
515 vertical growth (**Figure 5E-F**). All three elicitors were found to affect the expression
516 of several assayed CK and GA pathway genes (**Figs. 3, S5**), supporting the notion
517 that activating ISR or SAR results in transcriptional reprogramming that can effect
518 changes in plant developmental programs, and result in increased growth.
519
520 *Defense priming competence and plant growth promotion competence have*
521 *overlapping pathways*
522 SAR depends on SA signaling, while ISR relies on the JA/ET circuit. While SA was
523 shown to be antagonistic to JA in defense signaling (Pieterse et al., 2012, 2009),
524 there are cases where SA and JA were reported to positively interact (De
525 Vleesschauwer et al., 2013; Ferrari et al., 2003; Liu et al., 2016; Ullah et al., 2022).
526 Dependence of ISR on SA signaling has been reported as well (Martínez-Medina et
527 al., 2013). Distinctions between ISR and SAR are not clear cut in tomato, and overlap
528 between the two was reported (Betsuyaku et al., 2018; Liu et al., 2016).
529 Interestingly, though *Trichoderma* spp. are known to potentiate increases in
530 defense via the JA/ET pathway, T39 activated the SA-dependent *PR1a* (Meller Harel
531 et al., 2014). Furthermore, *B. cinerea* itself, a necrotrophic fungal pathogen against
532 which the plant host requires intact JA signaling to defend itself (AbuQamar et al.,
533 2008), can also activate the SA pathway (Meller Harel et al., 2014). To examine
534 whether the same pathways required for defense in response to priming agents are
535 required for plant growth promotion, we used the SA deficient transgenic line
536 *NahG*, the reduced JA mutant *spr-2*, and the reduced ET sensitivity mutant *neveripe*

537 (*nr*). As expected, ASM was not able to induce resistance in *NahG*, but remained
538 able to do so in *nr* and *spr-2*, while T39 induced resistance in *NahG*, but not in *spr-2*
539 or *nr*. 4C lost the ability to induce disease resistance in all three mutants (**Fig. 6A-C**).
540 Investigating the growth promoting effects in parallel, we observed similar results:
541 T39 promoted growth and increased agricultural and developmental parameters in
542 *NahG*, but was not able to do so in *spr-2* or *nr,* while ASM increased plant height in
543 *spr-2* and *nr*, but not in *NahG*, and 4C could not promote growth, development, or
544 agricultural parameters- in any of the mutant lines (**Fig.s 6D-F, 7, S8-S10**). The
545 background lines behaved as expected. These results support the notion that
546 development-defense tradeoffs can be uncoupled, with priming for defense
547 resistance also serving to promote plant growth, suggesting that components of
548 these two pathways may overlap.
549

550 *An overlapping functional "toolbox" is employed in defense and developmental*
551 *pathways*

552 In a meta-analysis of genes previously defined as morphogenesis promoting (Israeli
553 et al., 2021), the plant-pathogen interaction pathway was found to be up-regulated
554 (**Fig. S11**). While is it tempting to speculate that defense pathways are activated in
555 plant tissues undergoing morphogenesis or growth, another alternative explanation
556 could be that genes with similar functions are required in both defense and
557 developmental processes, resulting in their grouping into similar functional
558 pathways. However, our results indicate that some cross-potentiation between
559 defense and developmental pathways seems likely. We propose that the
560 "development-defense" tradeoff concept should be updated to include the idea
561 that within a defined window, defense priming may actually serve to support
562 developmental processes, rather than inhibit them. **Fig. 8** provides a model
563 conceptualizing the involvement of immunity priming in the mitigation of growth-
564 defense trade-offs. When biotic stress is high, growth is naturally inhibited to free
565 up resources for the activation of stress responses and pathogen resistance
566 mechanisms. Pre-activation of ISR, which primarily requires the JA pathway and
567 promotes CK response, results in rescue of growth inhibition while promoting

568 defense. Pre-activation of SAR, which primarily requires the SA pathway and may
569 not substantially affect the CK/GA balance, promotes defense but results in a more
570 minor growth inhibition rescue as compared with ISR.

571 While plants in nature tend to be uniquely adapted to their particular habitat, and
572 can develop and complete their life cycle in the face of environmental and
573 pathogenic stressors, changing climate and intense cropping practices have in some
574 cases rendered such adaptations ineffective. Our work provides a framework for
575 further research towards the prevention of negative effects that increased
576 immunity can have on plant development and growth. Pending future research, this
577 could potentially be exploited agriculturally in certain cases.

578 **Materials and methods**

579 Plant materials and growth conditions

580 Tomato cultivars and mutant and transgenic lines were used throughout the study
581 as detailed in Table 1. Plants were grown from seeds in soil (Green Mix; Even-Ari,
582 Ashdod, Israel) in a growth chamber (seedling assays) under long day conditions (16
583 hr:8 hr, light: dark) at 24°C. Mature plants for disease and immunity assays and yield
584 experiments were grown in a 2mm nylon mesh nethouse (spring, summer, and fall-
585 ambient conditions) or in a glasshouse (winter, ambient light supplemented with
586 heating to 24°C).

587

588 **Table 1: Plant genotypes used in this study**

Genotype name	Source	References
S. lycopersicum cultivar M82		
S. lycopersicum cultivar Moneymaker (Mm)		
S. lycopersicum cultivar Castelmart (Cm)		
S. lycopersicum cultivar Pearson (Pn)		
pTCSv2::3×VENUS Overexpression of VENUS driven by the synthetic two-component signaling sensor pTCSv2. M82	Bar lab	(Bar et al., 2016; Steiner et al., 2020)

background line.		
Suppressor of the prosystemin-mediated response 2 "*spr-2*" - reduced JA production. Castlemart background line.	Prof. Bettina Hause	(Goetz et al., 2012; Montero-Vargas et al., 2018).
Decreased SA transgene "*NahG*", Moneymaker background line.	Bar lab	(Mehari et al., 2015).
Decreased ethylene sensitivity mutant *neverripe* ("*nr*"). Pearson background line.	Bar lab	(Mehari et al., 2015).
pBLS>>IPT7 ("*IPT*"): Transgenic overexpression of IsoPentenyl Transferase7 from the leaf *BLS* (Lifschitz et al., 2006) promoter. Elevated endogenous leaf levels of CK. M82 background line.	Prof. Naomi Ori	(Bar et al., 2016);(Bar et al., 2016; Shani et al., 2010).
pFIL>>CKX3 ("CKX")- Transgenic overexpression of Cytokinin Oxidase3 from the leaf *FIL* (Bonaccorso et al., 2012) *promoter*. Decreased endogenous leaf CK content. M82 background line.	Prof. Naomi Ori	(Bar et al., 2016);(Bar et al., 2016; Shani et al., 2010).
clausa ("clau"): Recessive MYB TF mutant. High CK sensitivity coupled with low CK content; low GA sensitivity coupled with increased amounts of pre-active GAs. M82 background line.	Prof. Naomi Ori	(Bar et al., 2016; Israeli et al., 2021).
pFIL>>GFP-proΔ17: Transgenic overexpression of a mutated, un-processed version of the DELLA TF *Procera* from the leaf *FIL* promoter. Low GA signal. M82 background line.	Prof. David Weiss	(Nir et al., 2017).
procera: Recessive mutant in the DELLA TF *Procera*. ΔGRAS allele. High GA signal. M82 background line.	Prof. David Weiss	(Livne et al., 2015).

589

590 *pBLS>>IPT7*, "*IPT*", overexpresses the rate limiting enzyme in biosynthesis of the CK
591 isopentenyl driven from the leaf-specific *BLS* promoter (Lifschitz et al., 2006), and
592 has high CK levels (Márquez-López et al., 2019; Redig et al., 1996; Smigocki and
593 Owens, 1988). *pFIL>>CKX3, "CKX"*, overexpresses a CK degrading dehydrogenase
594 enzyme driven from the leaf specific *FIL* promoter (Bonaccorso et al., 2012), and has
595 low CK levels (Nishiyama et al., 2011; Reid et al., 2016). These lines were previously
596 characterized (Shani et al., 2010). *pFIL>>GFP-proΔ17* "*proΔ17*" overexpresses a
597 mutated DELLA protein that is constitutively active, driven from the *FIL* promoter,
598 resulting in inhibition of GA signaling (Nir et al., 2017). *procera* "*pro*" is a recessive
599 mutation in the single tomato *DELLA* gene *procera*, resulting in constitutive high GA
600 signaling (Livne et al., 2015). *clausa* "*clau*" is a recessive MYB transcription factor
601 mutant that has increased CK and reduced GA sensitivity (Israeli et al., 2021).
602 Promoter driver lines were selected to minimize pleiotropic effects, therefore,
603 *pBLS>>IPT* was used rather than *pFIL>>IPT*. The leaf developmental phonotypes of
604 *CKX* and *pro*, or *IPT*, *clau*, and *proΔ17* are relatively similar in terms of leaf
605 complexity (**Fig. S6**), suggesting that high CK and low GA, or low CK and high GA, can
606 have similar developmental effects, supporting the notion that the ratio between
607 these hormones, rather than their individual content, determine developmental
608 programs.
609
610 Elicitor treatments
611 For activation of SAR we used ASM (Marolleau et al., 2017), and for activation of ISR
612 we used *T. harzianum* isolate T39 (Elad, 2000b). *B. megaterium* 4C was considered
613 to likely activate both pathways (Gupta et al., 2021a). For disease and immunity
614 assays, elicitors were applied to 4-5-week-old plants by spraying the fourth and fifth
615 leaves to drip. Two treatments were given: 3 days before challenge, and 4 hours
616 before challenge. For agricultural parameters, elicitors were applied to 3-4-week-
617 old plants by soil drench, using 5 mL / pot of the indicated concentrations.
618 Treatments were applied once a week for four consecutive weeks. For
619 developmental analyses, gene expression and hormone content quantification,
620 elicitors were sprayed to drip on seedlings of the indicated ages. Two treatments

621 were given, one week apart, in all cases except for TCS visualization, in which one
622 treatment was applied.
623
624 ASM (Acibenzolar-S-Methyl), also known as BTH (Benzothiadiazole), a synthetic
625 analog of SA, is used commercially to enhance disease resistance (and marketed
626 under the trade name "Bion" (Marolleau et al., 2017; Oostendorp et al., 2001).
627 Previously, application of BTH reduced plant biomass and was correlated with
628 induction of SA-mediated defense responses (Huot et al., 2014). SA analogues have
629 been found to effectively reduce gray mold (*Botrytis cinerea*) disease in tomato
630 (Meller Harel et al., 2014). ASM was shown to be processed in vivo by an SA
631 processing enzyme (Tripathi et al., 2010). As ASM is known to be phytotoxic (Ishii et
632 al., 2019), to work with non-phytotoxic concentrations, we calibrated the system
633 using two different ASM concentrations (**Fig. S2**). 0.01% of ASM were found to
634 reduce seedling height and number of leaves produced (**Fig. S2A-B**). In 4-week-old
635 plants, 0.01% ASM reduced the number of side-shoots produced, but did not
636 negatively affect other plant growth parameters. Treatment with 0.001% of ASM
637 had no negative effects on plant growth (**Fig S2A-B, D-G**), promoted height in both
638 seedlings and 4-week-old plants (**Fig. S2A,D**), and also induced immunity to *B.*
639 *cinerea*, while 0.01% did not (**Fig. S2C**). Following calibration and verification of lack
640 of toxicity (**Fig. S2**), ASM was applied to plants at a concentration of 0.001% v/v.
641
642 *Trichoderma* spp. are established plant growth promoting fungi that enhance
643 disease resistance by inducing ISR in a JA/ET dependent manner (Meller Harel et al.,
644 2014). *Trichoderma* spp. have been shown to promote plant growth and activate
645 ISR against a broad spectrum of pathogens. They are ubiquitous filamentous fungi
646 that colonize the rhizosphere and phyllosphere, promote plant growth, and
647 antagonize numerous foliar and root pathogens (Gupta and Bar, 2020). *T.*
648 *harzianum* isolate T39 was cultured on PDA plates (Difco lab) at 25°C in natural
649 light. Conidia were harvested in water and applied to plants at a concentration of
650 10^7 conidia/ mL.
651

652 *Bacilli* spp. are known to promote growth and induce immunity (Miljaković et al.,
653 2020). *B. megaterium* 4C is a phyllosphere isolate we previously demonstrated
654 induces immunity and promotes growth and development of mature plants and
655 seedlings (Gupta et al., 2022a, 2021a). Overnight (Luria-Bertani medium) cultures of
656 *B. megaterium 4C* were washed twice in sterile distilled water, and re-suspended in
657 DW. The cell suspension was adjusted to an optical density of OD_{600}=0.1
658 (approximately equal to 10^8 CFU/ mL), and applied to plants via either foliar spray
659 or soil drench as indicated.
660
661 Measuring growth and agricultural parameters
662 Plants were treated with T39 or ASM once a week by soil drench for 4 weeks,
663 starting from when the plants reached three unfurled leaves, typically 10-14 days
664 after germination. The first three treatments were given at a volume of 5 ml, and at
665 last treatment was given at a volume of 10 ml. Growth parameters were measured
666 once a week: shoot length was measured using a hand ruler from root crown to
667 shoot apical meristem. The number of side shoots was counted taking into account
668 only shoots of at least 5 cm in length. For leaf complexity, we counted the primary,
669 secondary and intercalary leaflets on the indicated leaf. Primary leaflets are
670 separated by a rachis, and some of them develop secondary and tertiary leaflets.
671 Intercalary leaflets are lateral leaflets that develop from the rachis later than the
672 primary leaflets and between them, and typically have a very short petiolule.
673 The following parameters were measured for yield: weight of fruits, weight of
674 shoot, calculated harvest index (yield weight/total plant weight (shoot + fruit
675 weight), and fruit soluble sugar content, measured as Brix %, using a ref-85
676 Refractometer (MRC).
677
678 Developmental analysis of seedlings
679 Seedlings were sprayed to drip with elicitors ten days after germination, and 17
680 days after germination. Growth and development parameters were analyzed one
681 week after each treatment. Parameters analyzed include height, fresh weight,
682 number of unfurled leaves, leaf developmental stage (No. of the leaf that is

683 Plastochron 1), formation of floral meristem, and leaf complexity. Shoots were
684 dissected with a surgical blade and No.5 forceps. Analyses following dissection were
685 conducted using a Nikon SMZ-25 binocular stereomicroscope or a Dino-Lite hand
686 held microscope.

687

688 *B. cinerea* infection and disease monitoring

689 Pathogenesis assays were conducted on detached leaves, with the exception of the
690 assays conducted with the defense mutants and their background lines (**Fig. 6**), in
691 which whole plants were inoculated. We have previously demonstrated that in
692 most cases in tomato, relative disease levels are similar on detached leaves and
693 whole plants, though the disease develops faster in detached leaves (Gupta et al.,
694 2020). In the case of the defense mutants, assays were conducted on whole plants,
695 since the similarity of detached leaf and whole plant assays has not been verified in
696 all the employed mutant genotypes. *B. cinerea* isolate *Bc*I16 cultures were
697 maintained on potato dextrose agar (PDA) (Difco Lab) plates and incubated at 22 °C
698 for 5-7 days. 0.4 cm diameter discs were pierced from 5 day old mycelia plates and
699 used for inoculation of both leaves and whole plants. Leaves and plants were kept
700 in a humid chamber following inoculation. The area of the necrotic lesions was
701 measured 3-5 days post inoculation using ImageJ software.

702 Ethylene measurement

703 Ethylene production was measured as previously described (Leibman-Markus et al.,
704 2017). Leaf discs 0.9 cm in diameter were harvested from plants treated as
705 indicated, and average weight was measured for each plant. Discs were washed in
706 water for 1-2 h. Every six discs were sealed with rubber septa in 10-mL Erlenmeyer
707 flasks, containing 1 ml assay buffer (10 mM MES pH 6.0, 250 mM sorbitol) with or
708 without the fungal elicitor ethylene inducing xylanase (EIX), 1 µg/mL, overnight at
709 room temperature. Disposable syringes (5 mL) with 21 gage needles were used to
710 extract the gaseous sample, and rubber stoppers were used to hold the sample until
711 it was injected into the Gas chromatograph. Ethylene content was measured by gas
712 chromatography (Varian 3350, Varian, California, USA).

713

714 Measurement of ROS generation

715 ROS was measured as previously described (Leibman-Markus et al., 2017). Leaf discs
716 0.5 cm in diameter were harvested from indicated treatments. Discs were floated in
717 a white 96-well plate (SPL Life Sciences, Korea) containing 250µl distilled water for
718 24 h at room temperature. After incubation, water was removed, and 50 µL ddH2O
719 water were immediately added to each well to prevent the tissue from drying up.
720 The ROS measurement reaction contained: Luminol 150 µM, HRP 15 µg/mL and
721 either 1µM flg22 or 1µg/ mL EIX. Light emission was measured immediately and
722 over the indicated time using a microplate reader luminometer (Spark, Tecan,
723 Switzerland).

724

725 Imaging of the CK-response synthetic promoter *pTCSv2*::3×VENUS

726 Stable transgenic M82 tomato *pTCSv2*::3 × VENUS plants that express VENUS driven
727 by the synthetic two-component signaling sensor pTCSv2 (Bar et al., 2016; Steiner
728 et al., 2020) were sprayed with elicitors ten days after germination. VENUS
729 expression was analyzed 48 h after treatment using a Nikon SMZ-25
730 stereomicroscope equipped with a Nikon-D2 camera and NIS Elements v. 5.11
731 software. ImageJ software was used for analysis and quantification of captured
732 images.

733

734 RNA extraction and qRT-PCR

735 Plants were sprayed with elicitors twice: ten days after germination, and 1 week
736 later. Plant tissue was collected 48 hours after each treatment. For source leaves, L2
737 and L3 were harvested from four different plants for each biological replicate. 16
738 plants were sampled in total. For sink tissue, ~70 mg of shoot apices (typically
739 consisting of the meristem and 5 youngest leaf primordia, m+5) were collected. 5-7
740 shoots were sampled per biological replicate, for a total of at least 40 plants. Total
741 RNA was extracted from tissues using Tri-reagent (Sigma-Aldrich) according to the
742 manufacturer's instructions. RNA (3µg) was converted to first strand cDNA using

743 reverse transcriptase (Promega, United States) and oligo-dT. qRT-PCR was
744 performed according to the Power SYBR Green Master Mix protocol (Life
745 Technologies, Thermo Fisher, United States), using a Rotor-Gene Q machine
746 (Qiagen). Relative expression was quantified by dividing the expression of the
747 relevant gene by the geometric mean of the expression of three established
748 normalizers: ribosomal protein *RPL8* (Solyc10g006580), Cyclophillin *CYP*
749 (Solyc01g111170) (Mascia et al., 2010; Mehari et al., 2015), and EXPRESSED *EXP*
750 (Solyc07g025390) (Bar et al., 2016). These genes were previously demonstrated to
751 possess stable expression levels in immunity or disease challenged tomato plants
752 (Gupta et al., 2021b; Lacerda et al., 2015; Mascia et al., 2010; Mehari et al., 2015;
753 Pizarro et al., 2020). Although the used normalizers are well established, we
754 employed three normalizers to prevent possible effects of lack of stability of any
755 one gene. The geometric mean, rather than arithmetic mean, of the expression
756 levels of these genes was used, in order to account for the possibility that their
757 expression levels are not independent of each other. All primers pairs had
758 efficiencies in the range of 0.97-1.03. Primers used for qRT-PCR are detailed in
759 **Supplemental Table 2**. The genes presented in Figures 3 and S4 are part of a larger
760 subset of genes tested. The full set of tested genes, and the outcome, are provided
761 in **Supplemental Table 3**.

762

763

764

765 Quantification of CK and GA in leaf tissues

766 *S. lycopersicum* cv M82 Tomato seedlings were sprayed with elicitors twice, at 10
767 and 17 days of age. 48 h after the second treatment, source leaves (L2-L3) and sink
768 leaves (shoot apices) were harvested. Hormone extraction was performed
769 according to (Shaya et al., 2019). Briefly, frozen tissue was ground to a fine powder
770 using a mortar and pestle. One gram of the powder was transferred to a 2 mL tube
771 containing 1 mL extraction solvent (ES) mixture (79% IPA: 20% MeOH: 1% acetic
772 acid) supplemented with 20ng of each deuterium-labelled internal standard (IS,

773 Olomouc, Czech Republic). The tubes were incubated for at 4°C 60min, with rapid
774 shaking, and then centrifuged at 14,000g for 15min at 4°C. The supernatant was
775 collected and transferred to 2mL tubes. 0.5mL of ES was added to the pellet and the
776 extraction steps were repeated twice. The combined extracts were evaporated
777 using speed-vac. Dried samples were dissolved in 200 µl of 50% methanol and,
778 filtered through a 0.22 µm syringe filter with a cellulose membrane. 5–10 µL were
779 injected for each analysis. LC–MS-MS analyses were conducted using a UPLC-Triple
780 Quadrupole MS (WatersXevo TQMS). Separation was performed on a WatersAcuity
781 UPLC BEH C18 1.7 µm 2.1x100mm column, with a VanGuard pre-column (BEH C18
782 1.7 um 2.1x5mm). The mobile phase consisted of water (phase A) and acetonitrile
783 (phase B), both containing 0.1% formic acid in the gradient elution mode. The flow
784 rate was 0.3 ml/min, and the column temperature was kept at 35 °C. Acquisition of
785 LC–MS data was performed using MassLynx V4.1 software (Waters). Quantification
786 was done using the isotope-labeled internal standards (IS).

787

788 Data analysis

789 Data is presented as minimum to maximum values in boxplots, or as average ±SEM
790 in bar graphs. For Gaussian distributed samples, we analyzed the statistical
791 significance of differences between two groups using a two-tailed *t*-test, with
792 additional post hoc correction where appropriate: Welch's correction for *t*-tests
793 between samples with unequal variances, or Holm-Sidak's correction when multiple
794 *t*-tests were applied to a data set. We analyzed the statistical significance of
795 differences among three or more groups using analysis of variance (ANOVA).
796 Regular ANOVA was used for groups with equal variances, and Welch's ANOVA for
797 groups with unequal variances. Significance in differences between the means of
798 different samples in a group of three or more samples was evaluated using a post-
799 hoc test. Tukey's post-hoc test was used for samples with equal variances, when the
800 mean of each sample was compared to the mean of every other sample.
801 Bonferroni's post-hoc test was used for samples with equal variances, when the
802 mean of each sample was compared to the mean of a control sample. Dunnett's
803 post-hoc test was used for samples with unequal variances. For samples with non-

Gaussian distribution, we analyzed the statistical significance of differences between two groups using a Mann-Whitney U test, and the statistical significance of differences among three or more groups using Kruskal-Wallis ANOVA, with Dunn's multiple comparison post-hoc test as indicated. Gaussian distribution or lack thereof was determined using the Shapiro-Wilk test for normality. Statistical analyses were conducted using Prism9™.

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Competing interests

The authors declare no competing interests.

Author contributions

M.B. conceived and designed the study. A.S., M.L-M., R.G., I.M., D.R-D., Y.E. and M.B. formulated the methodology and carried out the experiments. A.S., M.L-M., D.R-D., R.G., Y.E. and M.B. analyzed the data. All authors contributed to the writing of the manuscript.

Data Availability Statement

The authors declare that the data supporting the findings of this study are available within the paper and its Supporting Information files. Raw data is available from the corresponding author upon reasonable request.

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Figure legends

Figure 1: Treatment with T39, ASM, and 4C reduces *B. cinerea* disease, induces immune responses, and promotes plant growth and agricultural parameters.

A-B *S. lycopersicum* cv M82 4-5-week-old Tomato plants were sprayed twice with elicitors: 3 days and 4 h before inoculation with 3-day-old *B. cinerea* mycelia. Mock- DDW tretment. Lesion area was measured 5 days after inoculation. Experiment was repeated 5 independent times. **A** Lesion area. **B** representative images, 5 days after inoculation.

C-D Ethylene production was measured alone (C) or with the addition of 1 µg / mL EIX (D), using gas-chromatography. **E** ROS production following flg22 treatment was measured using the HRP luminol method, and expressed in terms of total RLU (Relative Luminescence Units).

F-I *S. lycopersicum* cv M82 Tomato plants were soil-drenched with elicitors or DDW (Mock) once a week for four weeks. **F** Height of 60-day-old plants. **G** Average fruit weight per plant. **H** Yield per vegetative plant weight, expressed as Harvest Index. **I** Average tomato soluble sugars, expressed as Brix %.

Boxplots indicate inner-quartile ranges (box), outer-quartile ranges (whiskers), median (line),
all points shown. Asterisks indicate significance from mock treatment (A), and different letters indicate statistically significant differences among samples (C-H) in Welch's ANOVA with Dunnett's post hoc test (A, E, F), one-way ANOVA with Tukey's post hoc test (D, G), or an unpaired two-tailed t-test with Welch's correction (C, H-I). A: N = 82, $**p<0.01$; $****p<0.0001$. C: N=15, $p<0.05$. D: N>10, $p<0.047$. E: N>12, $p<0.0007$. F: N=8, $p<0.018$. G: N=9, $p<0.04$. H: N=10, $p<0.004$. I: N>14, ns=not significant.

Figure 2: Treatment with T39, ASM, and 4C affects seedling growth and development.

S. lycopersicum cv M82 Tomato seedlings were sprayed with elicitors or DDW (Mock) once (at 10 days of age, **A,C,E**) or twice (at 10 and 17 days of age, **B,D,F**). **A,B** Height. **C, D** Fresh weight. **E, F** Number of leaves.

Boxplots indicate inner-quartile ranges (box), outer-quartile ranges (whiskers), median (line),
all points shown. Asterisks indicate statistical significance from Mock treatment in one-way ANOVA with Bonferroni's post hoc test, A: N=14, p<0.0003; B: N=12, p<0.0063; C: N=12, p<0.013; D: N=10, p<0.04; E:N=12, p<0.0001, F: N=8, p<0.0047.

G-R representative images at 24 days of seedlings (**G-J**), a shoot with the two oldest leaves removed (**K-N**), and Leaf No. 5 (**O-R**), which was at the P5 stage in Mock and ASM (**O, Q**), and at the P6 stage in T39 and 4C (**P,R**). **K-R** were acquired using a Nikon SMZ25 stereomicroscope equipped with a Nikon DS-RI2 camera and NIS elements software. Bars: G (For G-J): 1cm, K-N: 100 µM, O-R 250 µM.

SAM= shoot apical meristem; FM= floral meristem; SYM= Sympodial meristem (the vegetative meristem that continues to produce leaves after transition to flowering). P1/2/3/4 indicates developmental plastochron.

In O-R, numbers indicate pairs of initiated leaflets. "L" indicates lobe.

Figure 3: Treatment with elicitors affects CK and GA pathway gene expression and hormone content in source leaves.

S. lycopersicum cv M82 Tomato seedlings were sprayed with elicitors or DDW (Mock) twice, at 10 and 17 days of age. RNA was prepared from the second and third leaves, 48 h after the second treatment. qRT-PCR relative expression was normalized to the geometric mean of the expression of 3 normalizer genes: *EXP (*Solyc07g025390*), CYP* (Solyc01g111170), and *RPL8* (Solyc10g006580). **A** Cytokinin biosynthesis *IPT* genes. **B** Cytokinin activation *LOG* genes. **C** Cytokinin degradation *CKX* genes. D Cytokinin response regulators (*TRRs*). **E** Gibberellin biosynthesis genes (*GA2Oox/ GA3ox*). **F** Gibberellin degradation genes (*GA2ox*). **G** Quantification of CK and GA derivatives. tZ- transZeatin, iP- isopentenyl, iPR- isopentenylriboside. GA4/7- Gibberellin 4/7. **H** Ratio of the expression of the defense genes *PR1a* and *LoxD*. Below 1 suggests that ISR is the primary pathway activated, above 1 suggests SAR activation.

Bars represent mean ±SE. A-F, H: N=5. A-F: Asterisks represent statistical significance from Mock treatment in two-tailed *t*-test with Welch's correction where appropriate (unequal variances), for each gene. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns- not significant. G: N=4. G-H: Different letters represent statistically significant differences among samples in a two-tailed *t*-test with Welch's correction where appropriate (unequal variances). G: p<0.05, H: p<0.0025.

Figure 4: Treatment with T39, ASM, and 4C activates cytokinin response

S. lycopersicum cv M82 Tomato seedlings expressing the cytokinin response marker *pTCSv2*::3XVENUS were sprayed with elicitors or DDW (Mock) at 10 days of age, and imaged after 48 h. **A** Representative shoots. "P" number indicates the developmental plastochron. **B** Mean fluorescent signal, expressed as % of the Mock signal (AFU- arbitrary fluorescent units). m+3= meristem and 3 youngest primordia; P4= fourth plastochron (fourth youngest leaf primordium); P5= fifth plastochron, (fifth youngest leaf

primordium). Leaves 1 and 2 were removed, such that the oldest primordium was L3. **C** Corrected total fluorescence (CTF= Integrated Density – (Area of selected cell X Mean fluorescence of background reading), in the indicated tissues. Measurements were taken using FIJI-ImageJ.

Boxplots indicate inner-quartile ranges (box), outer-quartile ranges (whiskers), median (line),
all points shown. Different letters indicate statistically significant differences among samples in one-way ANOVA with Tukey's post hoc test or in Welch's t-test, untagged letters for m+3, tagged letters for P4, double-tagged letters for P5. B: N>9, p<0.048; C: N>12, p<0.05.

Figure 5: Treatment with T39 and 4C, but not ASM, requires CK to promote growth and disease resistance.

A, C, E, G, I, K Tomato seedlings of *S. lycopersicum* cv M82 (A-B), the transgenic *pBLS>>IPT* (C-D), *pFIL>>CKX* (E-F), *pFIL>>proΔ17* (G-H), or the recessive mutants *procera* (I-J) or *clausa* (K-L), all in the M82 background, were sprayed with elicitors twice, at 10 and 17 days of age. Seedlings treated with DDW were used as mock. Genotypes with high CK or low GA are indicated in blue, genotypes with low CK or high GA are indicated in red. High/ low refers to content or signaling. See also Table 1. Height was measured 1 week after the second treatment.

B, D, F, H, J, L Four-week-old tomato plants of the indicated genotypes were sprayed twice with elicitors or DDW (Mock): 3 days and 4 h before inoculation with 3-day-old *B. cinerea* mycelia. Lesion area was measured 3 days after inoculation. Experiment was repeated 3 independent times.

Boxplots indicate inner-quartile ranges (box), outer-quartile ranges (whiskers), median (line),
minimum to maximum values. Asterisks indicate statistical significance from Mock treatment in one-way ANOVA with Bonferroni's post hoc test (A, C, E, G, I, K, J), or in Kruskal-Wallis ANOVA with Dunn's post hoc test (B, D, F, H, L). A: N=38. B: N=24. C: N=24. D: N=28. E: N=12. F: N=14. G: N=9. H: N=12. I: N=14. J: N=38. K: N=18. L: N=32. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns- not significant.

Figure 6: Treatment with T39, ASM, and 4C shows differential effects on disease reduction and development in defense pathway mutants.

S. lycopersicum plants of the cultivars Moneymaker (Mm) and the reduced SA transgene *NahG* (**A, D**), Castelmart (Cm) and the reduced JA mutant *spr-2* (**B, E**), and Pearson (Pn) and the reduced ET sensitivity mutant *neveripe* (*nr*) (**C, F**), were sprayed with elicitors or DDW (Mock) either twice, at 3 days and 4 h before *Botrytis cinerea* inoculation (A-C), or soil-drenched once a week for four weeks (D-F).

A-C Plants were inoculated with 3-day-old *B. cinerea* mycelia. Lesion area was measured 3 days after inoculation. Experiment was repeated 4 independent times.

D-F Height of 60-day-old plants.

Boxplots indicate inner-quartile ranges (box), outer-quartile ranges (whiskers), median (line),
all points shown. Different letters indicate statistically significant differences among samples in one-way ANOVA with Tukey's post hoc test (A-C), or Welch's ANOVA with Dunnett's post hoc test (D-F). A: N>20, p<0.042. B: N>20, p<0.045. C: N>30, p<0.043. D: N> 5, p<0.045. E: N>3, p<0.042. F: N>12, p<0.035.

Figure 7: Treatment with T39, ASM, or 4C shows differential effects on plant height in defense pathway mutant seedlings.

S. lycopersicum seedlings of the cultivars Moneymaker (Mm) and the reduced SA transgene NahG (**A**), Castelmart (Cm) and the reduced JA mutant *spr-2* (**B**), and Pearson (Pn) and the reduced ET sensitivity mutant *neveripe* (*nr*) (**C**), were sprayed with elicitors or DDW (Mock) twice, at 10 and 17 days of age. Height was measured 1 week after the second treatment.

Boxplots indicate inner-quartile ranges (box), outer-quartile ranges (whiskers), median (line),
all points shown. Different letters indicate statistically significant differences among samples in one-way ANOVA with Tukey's post hoc test. A: N>9, p<0.034. B: N>9, p<0.044. C: N>6, p<0.039.

Figure 8: Model conceptualizing the involvement of immunity priming in mitigating growth-defense trade-offs.

Plants balance growth/development and defense throughout their lives. When biotic stress is high, growth is naturally inhibited as a mechanism to control resource allocation and better activate immunity and pathogen resistance mechanisms. Pre-activation of ISR, which primarily requires the JA pathway and promotes CK response, results in rescue of growth inhibition while promoting defense. Pre-activation of SAR, which primarily requires the SA pathway and may not substantially affect the CK/GA balance, promotes defense but results in a more minor growth inhibition rescue as compared with ISR.

Growth indicated in green, defense indicated in brown/yellow. Illustration generated with Biorender.com.

ISR Induced Systemic Resistance

SAR Systemic Acquired Resistance

SA Salicylic Acid

JA Jasmonic Acid

CK Cytokinin

GA Gibberellin

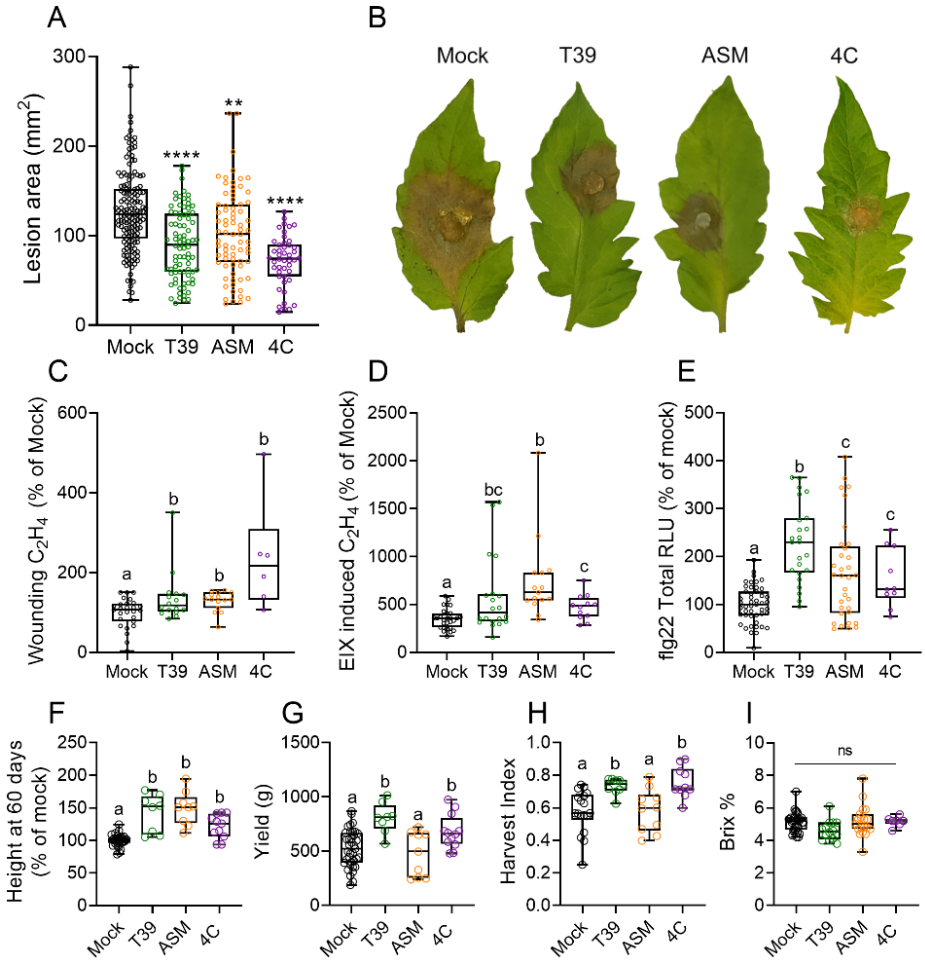

Figure 1: Treatment with T39, ASM, and 4C reduces *B. cinerea* disease, induces immune responses, and promotes plant growth and agricultural parameters.

A-B *S. lycopersicum* cv M82 Tomato plants aged 4-5 weeks were sprayed twice with elicitors: 3 days and 4 h before *Botrytis cinerea* inoculation. Plants were inoculated with 3 day-old *B. cinerea* mycelia. Plants treated with DDW were used as mock. Lesion area was measured 5 days after inoculation. Experiment was repeated 5 independent times. **A** Lesion area. **B** representative images, 5 days after inoculation.

C-D Ethylene production was measured alone (C) or with the addition of 1 µg / mL Ethylene Inducing Xylanase (EIX, D), using gas-chromatography. **E** ROS production following flg22 treatment was measured every 3 minutes for 60 minutes using the HRP luminol method, and expressed in terms of total RLU% (Relative Luminescence Units).

F-I *S. lycopersicum* cv M82 Tomato plants aged 3-4 weeks were soil-drenched with elicitors once a week for four weeks. Plants treated with DDW were used as mock. **F** Height of 60 day old plants. **G** Average fruit weight per plant. **H** The amount of yield obtained per vegetative plant weight, expressed as Harvest Index. **I** Average tomato soluble sugars, expressed as Brix %.

Boxplots are shown with inter-quartile-ranges (box), medians (line in box) and outer quartile whiskers, minimum to maximum values, all points shown. Asterisks indicate significance from mock treatment (A), and different letters indicate statistically significant differences among samples (C-H) in Welch's ANOVA with Dunnett's post hoc test (A, E, F), one-way ANOVA with Tukey's post hoc test (D, G), or an unpaired two-tailed t-test with Welch's correction (C, H-I). A: N = 82, ***p*<0.01; *****p*<0.0001. C: N=15, p<0.05. D: N>10, p<0.047. E: N>12, p<0.0007. F: N=8, p<0.018. G: N=9, p<0.04. H: N=10, p<0.004. I: N>14, ns=not significant.

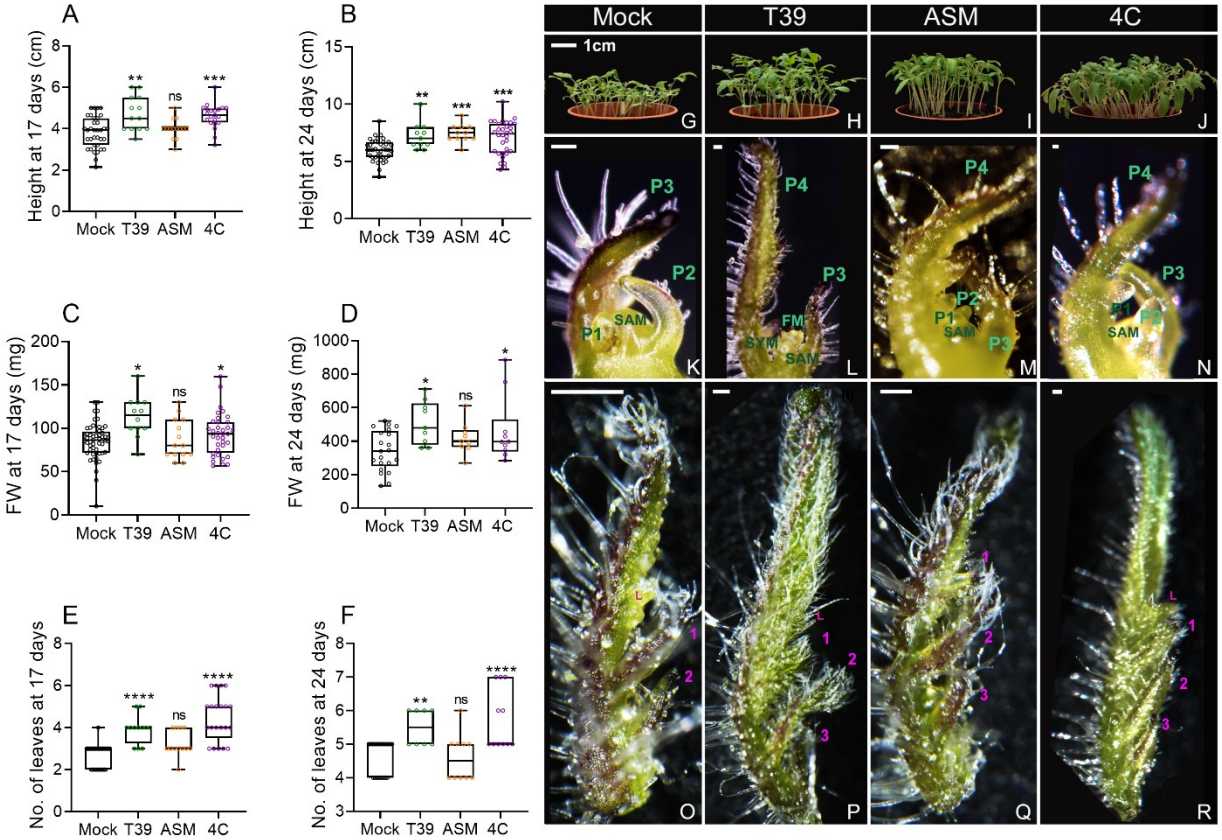

Figure 2: Treatment with T39, ASM, and 4C affects seedling growth and development.

S. lycopersicum cv M82 Tomato seedlings were sprayed with elicitors once (at 10 days of age, A,C,E) or twice (at 10 and 17 days of age, B,D,F). Seedlings treated with DDW were used as mock. **A,B** Height. **C, D** Fresh weight. **E, F** Number of unfurled leaves.

Boxplots are shown with inter-quartile-ranges (box), medians (line in box) and outer quartile whiskers, minimum to maximum values, all points shown. Asterisks indicate statistical significance from Mock treatment in one-way ANOVA with Bonferroni's post hoc test, A: N=14, $p<0.0003$; B: N=12, $p<0.0063$; C: N=12, $p<0.013$; D: N=10, $p<0.04$; E:N=12, $p<0.0001$, F: N=8, $p<0.0047$.

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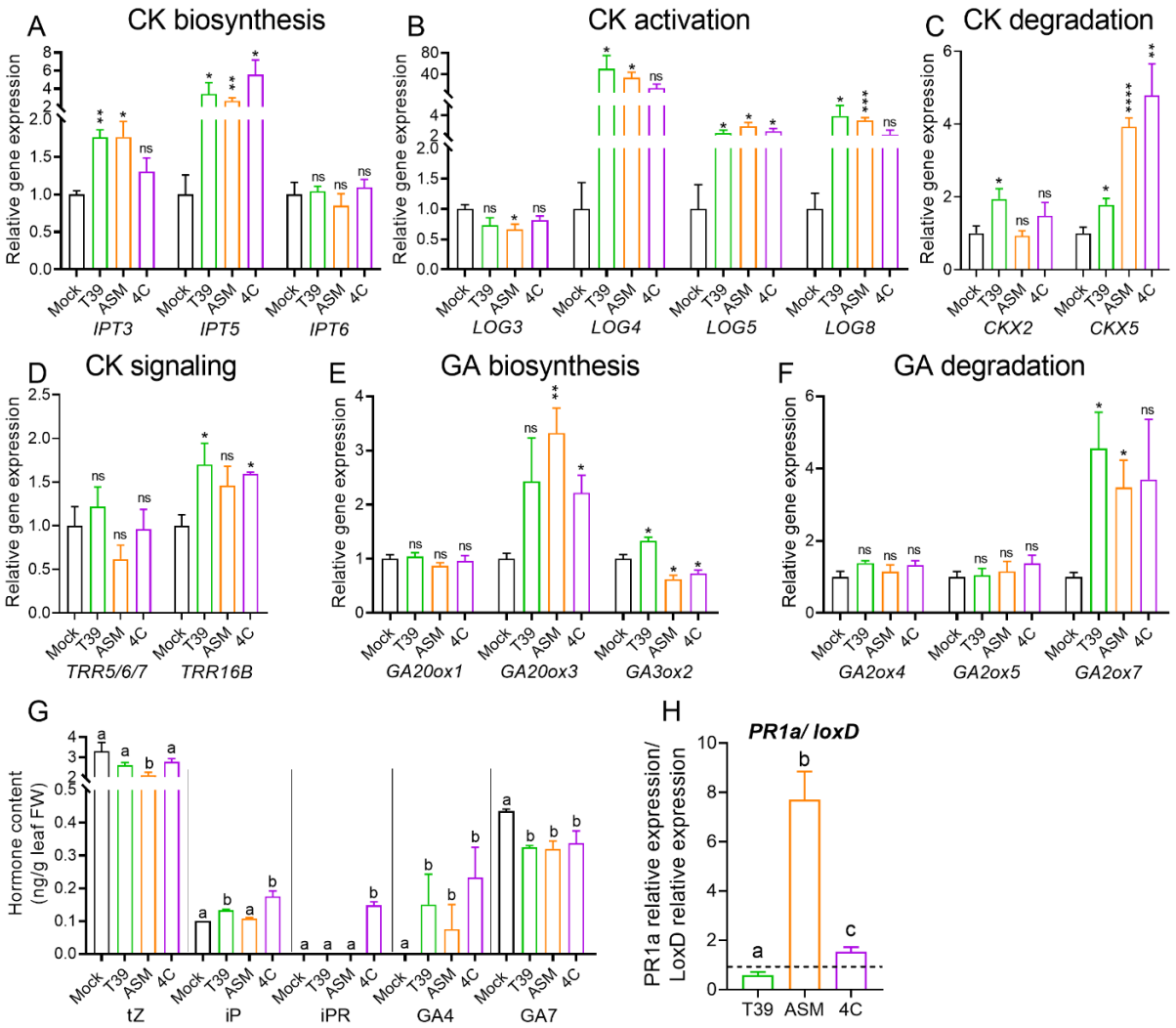

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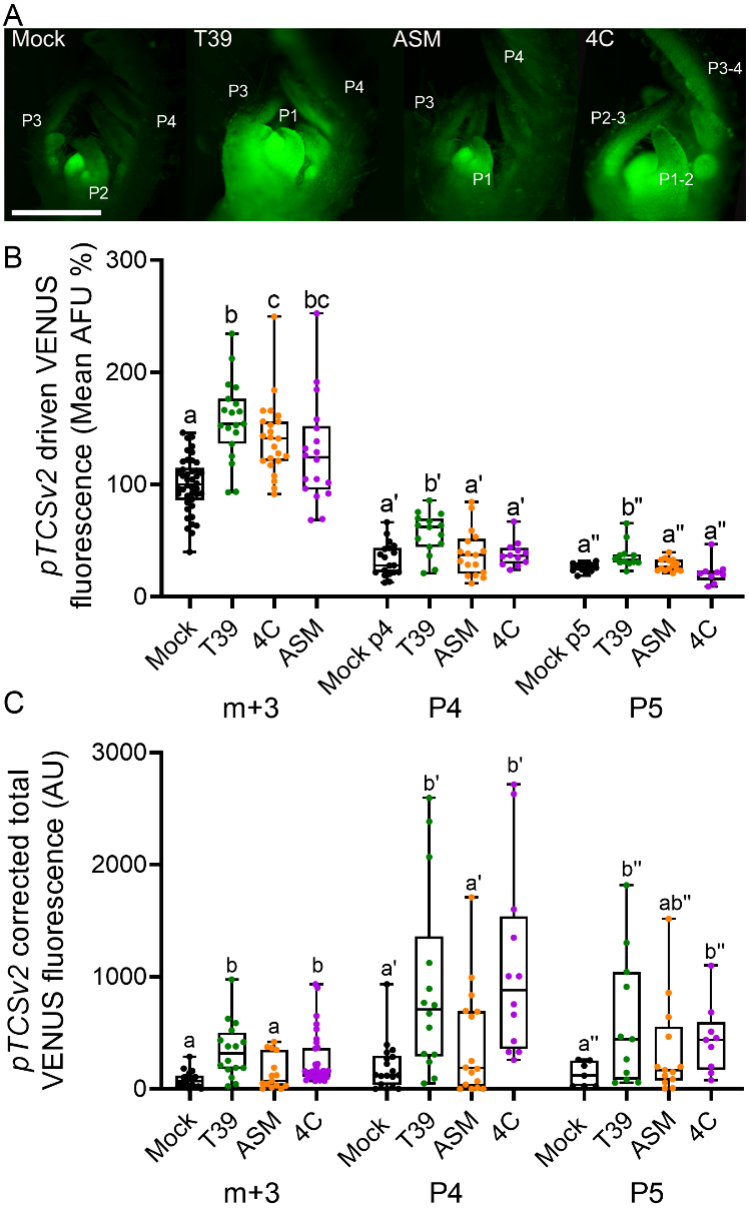

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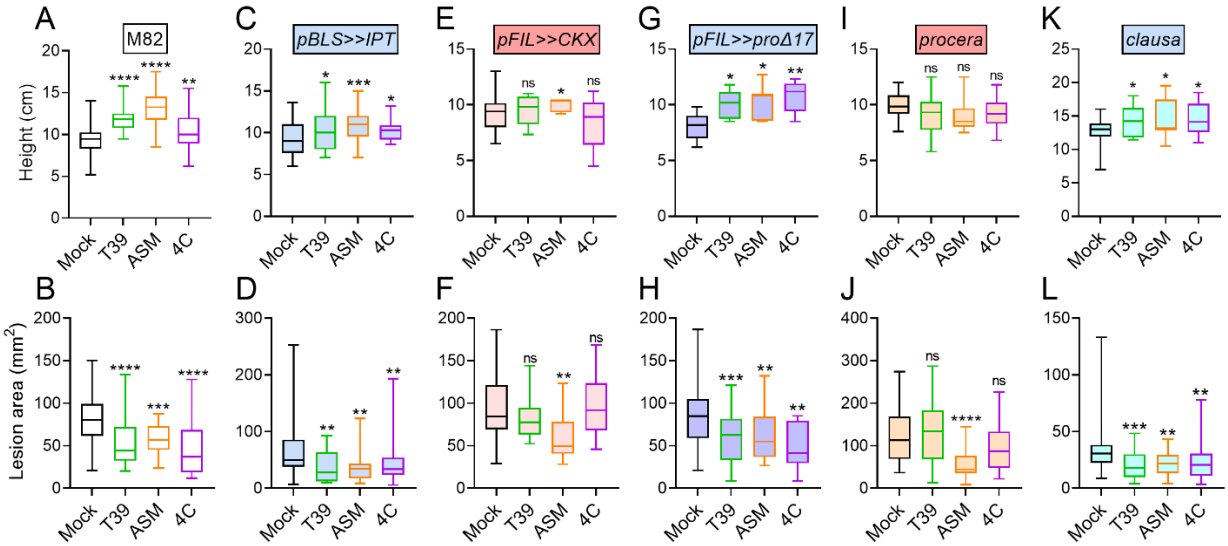

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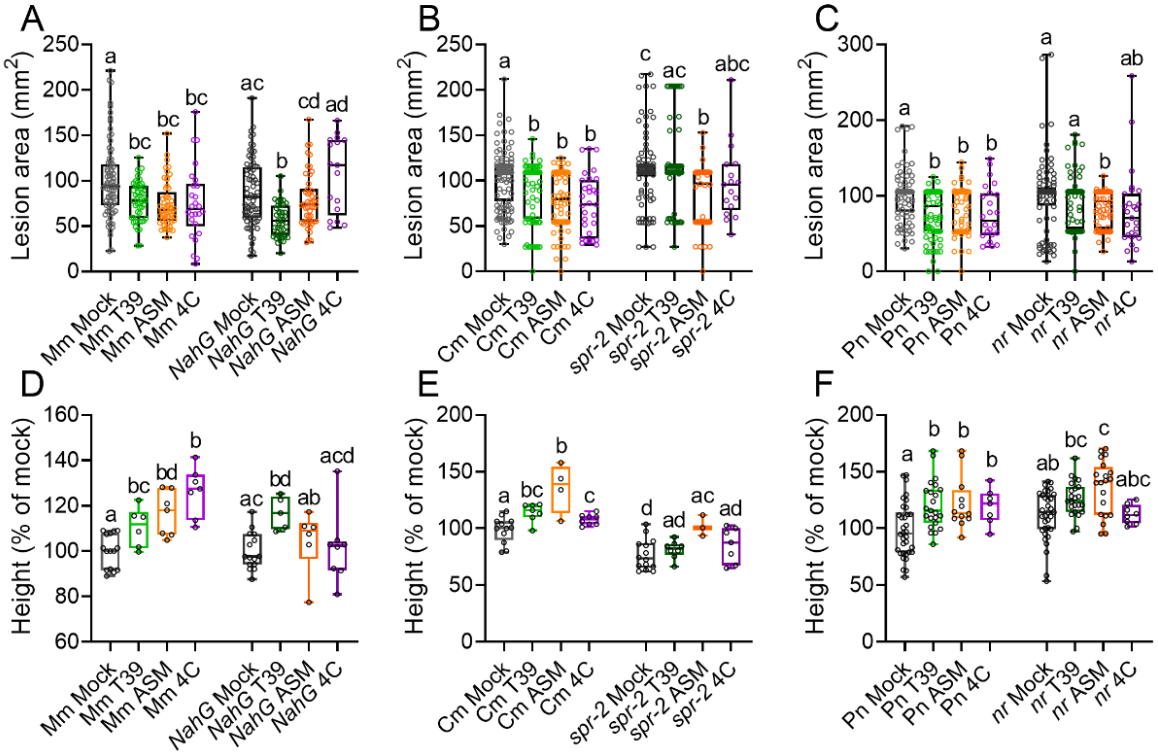


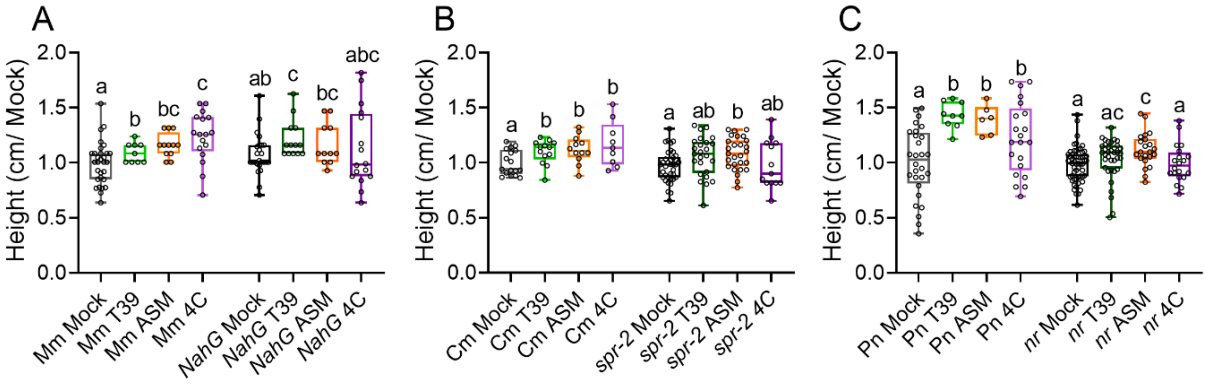
Figure 6: Treatment with T39, ASM, and 4C shows differential effects on disease reduction and development in defense pathway mutants.

S. lycopersicum plants of the cultivars Moneymaker (MM) and the reduced SA transgene NahG (**A, D**), Castelmart (Cm) and the reduced JA mutant *spr-2* (**B, E**), and Pearson (Pn) and the reduced ET sensitivity mutant *neveripe* (*nr*) (**C, F**), were sprayed with elicitors either twice, at 3 days and 4 h before *Botrytis cinerea* inoculation (A-C), or soil-drenched once a week for four weeks (D-F).

A-C Plants were inoculated with 3 day-old *B. cinerea* mycelia. Plants treated with DDW were used as mock. Lesion area was measured 3 days after inoculation. Experiment was repeated 4 independent times.

D-F Height of 60 day old plants.

Box plots indicate inner quartile ranges (box), outer quartile ranges (whiskers), median (line), all points shown. Different letters indicate statistically significant differences among samples in one-way ANOVA with Tukey's post hoc test (A-C), or Welch's ANOVA with Dunnett's post hoc test (D-F). A: N>20, p<0.042. B: N>20, p<0.045. C: N>30, p<0.043. D: N> 5, p<0.045. E: N>3, p<0.042. F: N>12, p<0.035.



<u>Figure 7: Treatment with T39, ASM, or 4C shows differential effects on plant height in defense pathway mutant seedlings.</u>

S. lycopersicum seedlings of the cultivars Moneymaker (Mm) and the reduced SA transgene NahG (**A**), Castelmart (Cm) and the reduced JA mutant *spr-2* (**B**), and Pearson (Pn) and the reduced ET sensitivity mutant *neveripe* (*nr*) (**C**), were sprayed with elicitors twice, at 10 and 17 days of age. Seedlings treated with DDW were used as mock. Height was measured 1 week after the second treatment.

Boxplots are shown with inter-quartile-ranges (box), medians (line in box) and outer quartile whiskers, minimum to maximum values, all points shown. Different letters indicate statistically significant differences among samples in one-way ANOVA with Tukey's post hoc test. A: N>9, p<0.034. B: N>9, p<0.044. C: N>6, p<0.039.

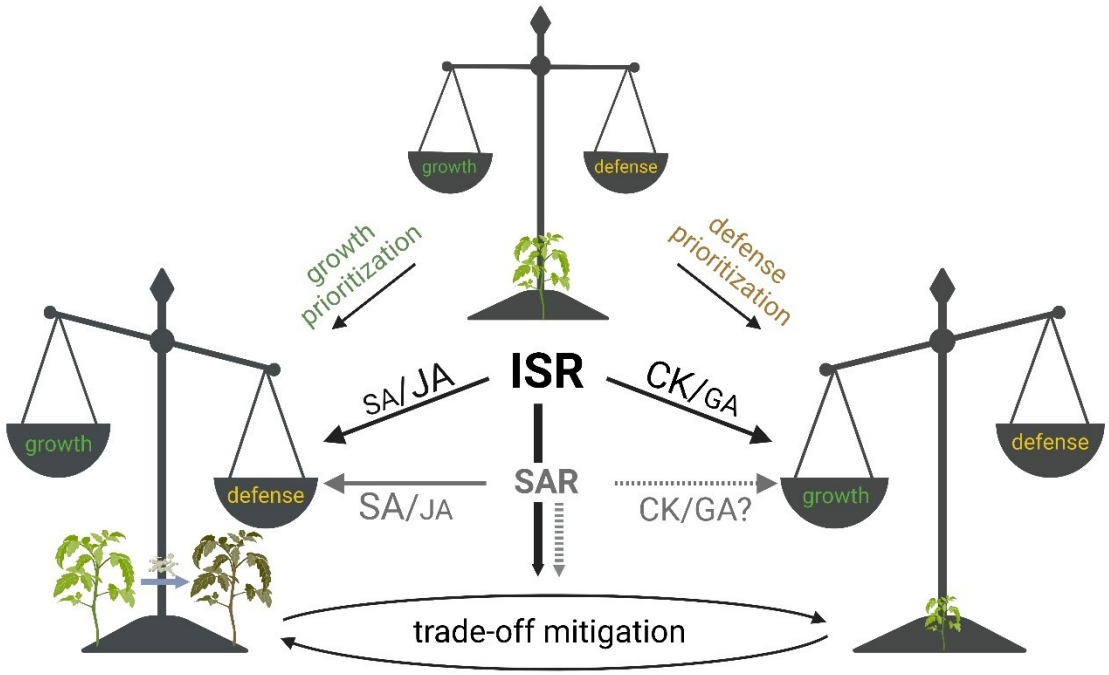


Figure 8: Model conceptualizing the involvement of immunity priming in mitigating growth-defense trade-offs.

Plants balance growth/development and defense throughout their lives. When biotic stress is high, growth is naturally inhibited as a mechanism to control resource allocation and better activate immunity and pathogen resistance mechanisms. Pre-activation of ISR, which primarily requires the JA pathway and promotes CK response, results in rescue of growth inhibition while promoting defense. Pre-activation of SAR, which primarily requires the SA pathway and may not substantially affect the CK/GA balance, promotes defense but results in a more minor growth inhibition rescue as compared with ISR.

Growth indicated in green, defense indicated in brown/yellow. Illustration generated with Biorender.com.

ISR Induced Systemic Resistance

SAR Systemic Acquired Resistance

SA Salicylic Acid

JA Jasmonic Acid

CK Cytokinin

GA Gibberellin