

Pure

Scotland's Rural College

Hull to caryopsis adhesion and grain skinning in malting barley: identification of key growth stages in the adhesion process

Hoad, SP; Brennan, M; Wilson, GW; Cochrane, MP

Published in:
Journal of Cereal Science

DOI:
[10.1016/j.jcs.2015.10.007](https://doi.org/10.1016/j.jcs.2015.10.007)

First published: 01/01/2016

Document Version
Peer reviewed version

[Link to publication](#)

Citation for published version (APA):

Hoad, SP., Brennan, M., Wilson, GW., & Cochrane, MP. (2016). Hull to caryopsis adhesion and grain skinning in malting barley: identification of key growth stages in the adhesion process. *Journal of Cereal Science*, 68, 8 - 15. <https://doi.org/10.1016/j.jcs.2015.10.007>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 **Hull to caryopsis adhesion and grain skinning in malting barley: Identification of key**
2 **growth stages in the adhesion process**

3

4 Stephen P. Hoad*, Maree Brennan, Gordon W. Wilson, Patricia M. Cochrane

5

6 Crop and Soil Systems Research Group, SRUC, West Mains Road, Edinburgh EH9 3JG

7

8 *Corresponding author. Crop and Soil Systems Research Group, SRUC, West Mains Road,
9 Edinburgh EH9 3JG United Kingdom. Tel +44 131 535 4342 email steve.hoad@sruc.ac.uk

10

11 **ABSTRACT**

12 Strong adhesion between the hull and the caryopsis is essential for barley to be of good
13 malting quality. Poor hull adhesion, a condition known as grain skinning, is undesirable for
14 malting and downstream processes. At present, the processes mediating hull adhesion during
15 grain development are poorly understood. The barley cultivar Chariot was grown in
16 greenhouse conditions and grain development was recorded at defined growth stages to
17 examine the timing of hull adhesion. Initiation of adhesion was first observed when caryopsis
18 fresh weight and volume were approaching their maximum at 19 days after anthesis, during
19 early dough. Hull adhesion was complete by 27 days after anthesis, or soft dough. Sections of
20 developing grains were observed using light and transmission electron microscopy to
21 examine a lipid-rich cementing layer believed to be responsible for adhesion between the hull
22 and the pericarp. Evidence for a lipid-rich cementing material was supported by the
23 observation that neither pectinase nor cellulase effected hull loosening. Grain growth, the
24 presence of globular material originating from the pericarp and an electron dense material in

25 the cementing layer are discussed in relation to hull adhesion. Grain skinning could be caused
26 by poor adherence of cuticular material or inadequate fusion between cuticles.

27

28 **Key Words**

29 Hull adhesion; Grain skinning; Cementing layer; Malting barley

30

31 **Abbreviations**

32 cl, cementing layer; daa, days after anthesis; EDTA, ethylene diamine tetra-acetic acid; GS,
33 Growth Stage; ncl, nucellar cuticle; rc, reticulation; tcl, testa cuticle; TEM, transmission
34 electron microscopy;

35

36 **1. Introduction**

37 Intact grains of barley (*Hordeum vulgare* L.) have an adherent outer coat or hull enclosing the
38 caryopsis. The malting industry has long considered good hull adhesion to be a highly
39 desirable trait for malting barley varieties (Roumeliotis et al., 2001). Detachment of the hull
40 from the caryopsis due to poor adhesion is called "skinning" (also known as "peeling"), and
41 for many reasons is undesirable for malting and downstream processes in brewing and
42 distilling. Barley grains without hulls will imbibe water and germinate more rapidly than
43 those with firmly adhering hulls, and their presence in a batch of malting barley results in
44 uneven malting through over- or under-modification of the starch of skinned grains (Agu et
45 al., 2002, 2008; Bryce et al., 2010; Roumeliotis et al., 1999). Grains without hulls are also
46 more likely to sustain physical damage which may harm the embryo and prevent germination
47 altogether (Agu et al., 2002; Olkku et al., 2005; Roumeliotis et al., 2001). During kilning,
48 peat can be added to the heat source and phenolic compounds are adsorbed onto the hull, thus
49 contributing important flavour compounds to malt whisky. After malting, the hull retains the

50 modified starch in a parcel which is not only convenient for transport, but can improve
51 storage (Roumeliotis et al., 1999; Olkku et al., 2005). Additionally, most brewing plant in
52 current use is optimised for covered barley, requiring the hull to filter the wort during
53 processing (Agu et al., 2008; Roumeliotis et al., 1999). Barley is therefore rejected at a
54 maltings if it contains an unacceptable proportion of grains that have skinned.

55

56 The hull is made up of two glumes, the lemma on the dorsal side and the palea on the ventral
57 side. Both glumes adhere to the surface of the pericarp (the outer layer of the caryopsis)
58 except at the distal end where the awn extends from the lemma, and along their somewhat
59 hyaline edges where the lemma usually overlaps the palea. A cementing material, described
60 as a sticky substance formed in the outer layers of the caryopsis, was reported to be
61 responsible for hull adhesion (Harlan, 1920). Cochrane and Duffus (1979) found that this
62 layer could be stained with the lipophilic dye Sudan IV. Gaines et al. (1985) did not detect
63 protein or carbohydrate in this material but showed that it was rich in lipid. More recently, it
64 was concluded that the cementing layer between the hull and pericarp was lipid rich as it
65 stained with Oil Red O (Olkku et al., 2005) and Sudan Black B (Taketa et al., 2008). Gaines
66 et al. (1985) used transmission electron microscopy in a comprehensive study of the
67 cementing layer during grain development. They found that in mature plants, the thickness of
68 the cementing layer was highly variable among different grains (130 to 600 nm), although
69 there was little variation in thickness throughout a single grain.

70

71 In naked barley, in which the caryopsis naturally threshes free of the entire hull, the cuticle
72 present on the pericarp was much thinner than that in hulled barley at only 35 to 50 nm
73 (Gaines et al., 1985). The naked phenotype has been shown to be controlled by the *Nud* gene,
74 a homolog of the Arabidopsis WIN1/SHIN1 ethylene response factor which regulates cutin

75 biosynthesis (Taketa et al., 2008). Naked barley does not produce the cementing material
76 responsible for hull adhesion (Gaines et al., 1985; Taketa et al., 2008), and the phenotype is
77 distinct from that of covered barley in which skinning occurs. In grain exhibiting skinning the
78 quality of the cementing material is apparently compromised and typically partial hull loss is
79 observed. Olkku et al. (2005) observed that hull-caryopsis separation could occur through
80 breakage of epidermal cells or thin-walled cells within the hull, rather than separation along
81 the cementing layer. They proposed that the physical structure of the hull was therefore the
82 influential factor in the quality of hull adhesion.

83

84 Earlier work has described the structure of the cementing layer at days after anthesis, but not
85 as grain developmental stages. This omission needs to be addressed to enable genotypic and
86 environmental causes of grain skinning to be explored. The objectives of this study were to
87 provide: i) a more precise definition of skinning in relation to well-defined developmental
88 phases, or growth stages and ii) a better understanding of the hull adhesion process in relation
89 to grain structure and development of the lipid-rich cementing layer. This is an essential
90 precursor towards phenotypic screening for genotypes with differential expression of grain
91 skinning and the targeting of work to improve grain quality in crop improvement
92 programmes.

93

94 **2. Materials and Methods.**

95 2.1. Growth of plants

96 The spring barley cultivar Chariot was chosen as a genotype noted for its moderate risk of
97 grain skinning as observed in field trials carried out at SRUC. Plants of Chariot were grown
98 in a glasshouse in which day/night temperatures were maintained at a minimum of 15°C/10°C
99 for an 18 h photoperiod. Natural daylight was supplemented with mercury vapour lamps so

100 that the minimum photosynthetically active radiation at plant ear level was $150 \mu\text{mol m}^{-2} \text{s}^{-1}$.

101 Growth stages (GS) referred to throughout the text are those defined by Tottman and Broad

102 (1987).

103

104 2.2. Grain growth

105 Ears on main stems and tillers were tagged at anthesis, which was determined by visual

106 assessment of dissected flowers. Four ears were sampled, at intervals of days after anthesis

107 (daa) according to well-defined developmental stages (Table 1). Five grains were removed

108 from the middle of one side of each ear. The palea and lemma of each grain were removed

109 and their lengths and widths measured using a micrometer accurate to 0.01 mm. Dry weight

110 of the pooled paleas and lemmas from each ear was determined after drying at 70°C for 48 h.

111 Pooled fresh and dry weights of the caryopses of these grains were measured. Moisture

112 content was expressed as the percentage of water in fresh material. Five additional grains

113 were removed from the middle of the opposite side of the same ear. The length and width of

114 each caryopsis was measured after removing the palea and lemma. The caryopses from each

115 ear were pooled and their volume was measured by displacement of water (by weight) using

116 a 5 cm^3 graduated flask. Mean values for each organ were calculated from the replicate

117 pooled samples.

118

119 Measurements of the separated paleas, lemmas and caryopses were made until 27 daa, after

120 which the hull components could not be removed from the caryopsis without damaging the

121 pericarp or underlying tissues. Thereafter, only the whole grain was measured. The hull

122 adherence phase was assessed by the ease of palea and lemma removal from the caryopsis at

123 regular intervals between 14 and 27 daa. Descriptive statistics (GenStat Release 15.1, VSN

124 International Ltd) were used to quantify change in growth and development of each organ:
125 with means and standard errors of the mean calculated at each growth stage.

126

127 2.3. Light microscopy using hand-cut sections

128 Ears on the main stems and tillers were tagged at anthesis. At 24, 38 and 45 daa grains were
129 sampled from the middle of the ears; these timings corresponded with grains in the soft to
130 hard dough stages or GS85-87 (Table 1). Hand-cut transverse sections taken from mid-grain
131 were collected in distilled water and then: (i) stained with Toluidine blue O 0.05% (w/v) in
132 sodium benzoate buffer pH 4.5 (Cochrane, 1985) and mounted on microscope slides using a
133 glycerol-gelatin mountant (Sigma-Aldrich), and examined using bright-field microscopy or,
134 (ii) stained in Fluorol Yellow 088 (Brundrett et al., 1991), and examined and photographed,
135 for fluorescent images as described previously (Cochrane et al., 2000).

136

137 2.4. Transmission electron microscopy

138 Transverse slices 1 mm thick were cut from the middle of grains harvested at 24 or 45 daa
139 (soft and hard dough stages) under a fixative containing 2.5% glutaraldehyde in 0.025 M
140 sodium phosphate buffer pH 7.2 (Cochrane, 1994). After immersion in the fixative for 4h at
141 room temperature, the tissue slices were dehydrated in an ethanol series of 40, 60, 80, 90, 95
142 and 100% (x 2, 15 min each concentration) and embedded in LR White resin. Sections of the
143 resin-embedded material were stained using uranyl acetate (used as a 1% aqueous solution at
144 pH 5.0) and lead citrate (0.1%), and examined in a Philips CM120 Biotwin transmission
145 electron microscope.

146

147 2.5. Enzyme treatments

148 Intact grains and transverse slices of grains harvested at 24 daa and 45 daa and cut at mid-
149 grain were incubated with and without gentle agitation, for periods up to two days at room
150 temperature or at 37°C in the following buffered enzyme solutions: 180 units cm⁻³ pectinase
151 (3.2.1.15, Sigma Aldrich, UK) in citric/phosphate buffer (pH 4.0); 25 units cm⁻³ cellulase
152 (3.2.1.4, Sigma Aldrich, UK) in citric/phosphate buffer (pH 5.0); 180 units cm⁻³ pectinase
153 and 25 units cm⁻¹ cellulase in citric/phosphate buffer (pH 5.0); 0.07 M sodium ethylene
154 diamine tetra-acetic acid (EDTA) in borate buffer (pH 8.5). As a control, samples of grains or
155 grain slices were incubated in the corresponding buffers without enzymes.

156

157 **3. Results**

158 3.1. Grain growth

159 Up to 18 days after anthesis (daa), between the late milk and early dough stages (GS77-83),
160 the hull did not adhere to the pericarp. Between 19 and 27 daa, when grains developed from
161 early to soft dough (GS83-85), hull-caryopsis adhesion strengthened and it was increasingly
162 difficult to remove the lemma and palea from the caryopsis without tearing the organs or
163 removing the epidermis of the pericarp. The hull could no longer be removed from the
164 caryopsis once grains had reached 27 daa, or soft dough stage. Hull adhesion was complete
165 when the caryopsis reached its maximum volume, and moisture content was 55% (Fig. 1a).
166 During the same period caryopsis fresh weight reached its maximum (Fig. 1b). Dry weight
167 accumulation was virtually linear between 14 to 37 daa and at the end of the adhesion phase
168 the caryopsis was 70% of its dry weight at maturity (Fig. 1b). Caryopsis length increased to a
169 maximum of 9.6 mm at 14 daa, late milk (GS77) and thereafter decreased to 7.8 mm at grain
170 maturity (Fig. 1c). Caryopsis width increased to 4.2 mm at 27 daa, soft dough (GS85) and
171 decreased gradually during grain maturation (Fig.1 c).

172

173 Both the lemma and palea increased in length between 4 to 19 daa. The full grown lemma
174 was 11.2 mm, whilst the shorter palea was 10.8 mm (Fig. 2a). From 4 daa, watery ripe
175 (GS71), to 27 daa (GS85), there was a small but significant increase in lemma width and a
176 small significant decrease in palea width (Fig. 2b). The lemma to palea width ratio increased
177 from 1.4:1 at 4 daa to 1.8:1 at 27 daa (Fig. 2b). Lemma dry weight was almost twice that of
178 the palea; the dry weight of both tissues increased up to 14 daa, late milk (GS77), with no
179 significant change thereafter (Fig. 2c).

180

181 3.2. Light microscopy

182 An example of good adhesion between the hull and the caryopsis at 24 daa, soft dough
183 (GS85), is shown in grain sections stained with Toluidine blue O (Fig. 3a). The cells of the
184 lemma inner epidermis (that is, the epidermal surface facing the caryopsis) have adhered to
185 the pericarp epidermal cells, which are characteristically compressed. In a grain sampled at
186 the same developmental stage, an example of skinning, where the hull has detached from the
187 pericarp, is shown in Fig. 3b. The inner epidermis of the lemma has detached from the
188 pericarp epidermis, which in this grain is not compressed. A gap between the hyaline edges
189 of the lemma and palea is evident in another grain sampled at 24 daa is shown in Fig. 3c. This
190 is where the hull is not sufficiently large enough to cover the caryopsis at its maximum
191 volume. The caryopsis is clearly exposed between the palea and the lemma, the ends of
192 which do not adhere to the surface of the pericarp. Detachment of a length of cells at the tip
193 of the lemma is shown in another grain harvested at 45 daa, hard dough (GS87) in Fig. 3d.

194

195 The lipid-specific stain Fluorol Yellow 088 stained three lipid-rich layers in a grain harvested
196 at 38 daa, soft to hard dough (GS85-87), (Fig. 4a). The innermost fluorescent layer is the

197 nucellar cuticle (ncl), just outside the aleurone. Some intracellular bodies in the aleurone cells
198 also stained in Fluorol Yellow 088. The middle stained layer was present above the testa (tcl)
199 and is difficult to distinguish from the innermost layer due to the strength of the fluorescence.
200 The outermost fluorescent layer was on the outer cell walls of the pericarp epidermis and it is
201 this layer we identify as the cementing layer (cl) between the hull and caryopsis. It was not
202 possible to distinguish between the cuticular layers of the outer pericarp and those of the
203 inner hull at this magnification. The lipid-rich layer between the palea and pericarp can be
204 seen along the ventral crease in another grain harvested at 45 daa, hard dough (GS87), (Fig.
205 4b). Here, the testa cuticular layer (tcl) is also seen (arrow).

206

207 3.3. Transmission electron microscopy

208 The greater resolution afforded by transmission electron microscopy (TEM) allowed better
209 comparison of the lipid-rich layers identified by staining with Fluorol Yellow 088. In a
210 section of grain harvested at 45 daa, hard dough (GS87), the cuticular cementing layer is
211 sandwiched between the thin-walled cells of the inner epidermis of the lemma and the
212 crushed cell walls of the pericarp (Fig. 5a, long arrow). A much thicker cuticular layer lies
213 between the pericarp and the crushed remains of the testa (medium arrow). Traces of another
214 thin lipid-rich layer lie outside the nucellus and aleurone (Fig. 5a, short arrow). The
215 cementing layer is approximately the same thickness as the nucellus cuticular layer and about
216 one-third of the thickness of the testa cuticular layer. A globular or "bubbly" structure at the
217 interface between the pericarp epidermal cell walls and the cuticle may represent production
218 of cuticular material in another section of grain harvested at 45 daa (Fig. 5b). Where there is
219 evidence of cuticular material being produced from the lemma, it is much less obvious
220 compared with the much higher production of material from the pericarp (Figs. 5b and 5c). In
221 Figs. 5b, 5c and 5e it is possible to see a double-line of electron dense material, running

222 parallel to the pericarp and lemma cell walls, in the otherwise uniform cuticular material
223 (arrowed). This interface is our hypothesised adhesion point within the cementing layer.
224 Reticulation that is a prominent feature of the cuticular layers of the testa and nucellus (Fig.
225 5d, denoted by 'rc') is absent from the pericarp cuticle (Fig. 5c). At an earlier stage of 24 daa,
226 early to soft dough (GS83-85), the cementing layer in another grain appears to have split
227 between the two cuticular layers of the pericarp and lemma (arrows), with filling of
228 additional cementing material (Fig. 5e, left side). At approximately 150 nm, the thickness of
229 the cementing layer was approximately the same at 45 daa as it was at 24 daa (Figs. 5c and
230 e).

231

232 3.4. Enzyme treatments

233 None of the enzyme treatments used on mature, immature, or sliced grains brought about the
234 separation of the hull from the caryopsis. Incubation in EDTA also failed to remove the entire
235 hull but some loosening did occur. This was evident in the ease with which it was possible to
236 pull away sections of the hull without bringing about complete detachment. When slices of
237 immature and mature grains were incubated in pectinase it was found that in the conditions
238 used, the starchy endosperm of the immature grains was completely disintegrated, and that of
239 the mature grains was partially disintegrated, but in both cases the hull remained firmly
240 attached to the pericarp. No disintegration of endosperm tissues was observed in control
241 slices.

242

243 **4. Discussion**

244 Temporal patterns of grain development will depend on environmental conditions, and
245 especially temperature, under which the plants are grown (Dupont and Altenbach, 2003).
246 Therefore, earlier studies which report the timing of hull adhesion as days after anthesis only

247 can be ambiguous in linking this process to stages of grain development or growth. For
248 example, Gaines et al. (1985) reported that in field-grown barley hull adhesion was initiated
249 by 10 days after anthesis when the pericarp and hull came into contact. Scott et al. (1983)
250 found that the hull could no longer be removed from the caryopsis when it had reached a dry
251 weight of 18 mg; from their graph of dry weight by days post-sowing (including anthesis),
252 this corresponded to approximately 16 days after anthesis. Both studies would indicate that
253 hull adhesion was initiated much earlier than reported herein, but unfortunately no further
254 description of grain development was given. The use of grain developmental stages allows
255 for the timing of processes such as hull adhesion to be interpreted in the context of growing
256 conditions of the plant and changes in both hull and caryopsis development. Such grain
257 assessment needs experienced operators, otherwise is it somewhat subjective, but the
258 procedure is essential for establishing the environmental and genetic causes of grain skinning,
259 and the means for its control.

260

261 Hull removal became difficult at the early dough stage (GS83) and adhesion was complete
262 before the end of the soft dough stage (GS85). In terms of grain growth, the period for
263 adhesion is near maximum caryopsis fresh weight and volume, at which time the hull has
264 reached its maximum size (Figs. 1 and 2). Changes in lemma and palea dimensions and
265 weight influence how well the hull covers the developing caryopsis. A gap between the
266 hyaline edges of the lemma and palea may be a common feature in grains at early dough.
267 However, this feature is distinct from "gape" between hull tissues of harvested grains, as it
268 often disappears as grain volume is reduced during ripening. True gape is when the lemma
269 and palea have not enveloped the caryopsis at harvest maturity. Variation in growth of the
270 lemma and palea could be important in determining how well a hull is matched to the
271 underlying caryopsis. For example, hull under-development can result in exposure of the

272 caryopsis as described by Hamachi et al. (1989, 1990). This mis-match between hull and
273 caryopsis growth would increase the risk of skinning if poor adhesion is further weakened by
274 a gap between the edges of the glumes. Our observation that lemma and palea growth
275 continues long after anthesis indicates that plasticity in growth of the hull could be a key
276 factor when considering variation in grain skinning among cultivars.

277

278 Skinned grains examined by light microscopy showed that separation between the hull and
279 caryopsis occurred in parallel with the outer surface of the pericarp to lemma boundary,
280 which is the location of the cementing layer. This was in contrast to breakage of epidermal
281 cells or thin-walled cells within the hull in skinned grains reported by Olkku et al. (2005), but
282 consistent with the earlier study by Gaines et al. (1985) demonstrating how separation of the
283 hull from the caryopsis occurred along the cementing layer, which typically remained
284 attached to the pericarp. Evidence from fluorescence microscopy of hand-cut sections of
285 fresh grains confirms that the cementing layer between the pericarp and the hull of barley
286 grains is largely, if not entirely, composed of lipid-rich material, as shown previously (Gaines
287 et al., 1985; Olkku et al., 2005).

288

289 Our observations from TEM indicate that the cementing layer contains two cuticles – the hull
290 and pericarp. Adhesion would thus be formed by the fusion of the two cuticles. The presence
291 of a double-line of electron dense layer inside the otherwise amorphous almost electron-
292 lucent cementing layer would seem to provide evidence of this fusion. We assume that prior
293 to the cuticles merging, each cuticle surface would have crystalline surface waxes typical of
294 plant cuticles (Jeffree, 1996), although their morphology and any changes that occur on
295 adhesion cannot be determined from this study.

296

297 The cementing layer has a well-developed ‘bubbly’ boundary with the pericarp epidermal cell
298 wall but lacks reticulation and is amorphous throughout. This uneven interface between the
299 cell wall and cuticle has the same structure as that of typical plant surface cuticles undergoing
300 a high level of cuticular material synthesis, which Jeffree (1996) interprets as evidence of the
301 deposition of globular masses of cutin. He concludes that this process takes place after the
302 formation of the cuticle proper. Deposition of cuticular material was much less evident at the
303 boundary with the inner epidermis of the glumes (e.g. lemma). Gaines et al. (1985) proposed
304 that it is possible that all cuticular material in the cementing layer originates in the pericarp
305 epidermis. Our interpretation is that both the pericarp and glumes contribute cuticular
306 material to the cementing layer. Prior to hull adhesion, the cells of the inner epidermis of the
307 glumes would at least produce a cuticle proper but may not proceed along the pathway of
308 cuticular membrane formation as described by Jeffree (1996). The thickness of the pericarp to
309 hull cementing layer was approximately the same at hard dough as it was at soft dough, thus
310 indicating that deposition of cuticular material ceased well before grain-filling was
311 completed.

312

313 The structure of the cementing layer was also somewhat different from that of the testa
314 cuticular layer. The testa cuticle lacked a ‘bubbly’ boundary and had a more reticulate
315 structure, indicating that its development was completed earlier in grain development. The
316 absence of striations within the cementing layer was distinct from the electron dense material
317 mentioned by Cochrane and Duffus (1979) and the cuticular lamellar structure described by
318 Freeman and Palmer (1984) and Gaines et al. (1985), which are typical of mature plant
319 cuticles (Jeffree, 1996).

320

321 When the hull separates from the caryopsis i.e. when skinning takes place, the cementing
322 layer is thought to separate from the hull and remain attached to the pericarp (Gaines et al.,
323 1985). Our observations suggest that the separation may occur along the electron-dense line
324 in the amorphous layer (Figure 5e). There would thus be two different interpretations of the
325 cause of skinning. It could be due to a failure of the cuticular material to adhere to the surface
326 of the inner epidermis of the glumes (palea or lemma), or it could be due to inadequate fusion
327 of the cuticle proper of the pericarp epidermis with that of the inner epidermis of the glumes.
328 Whichever is the cause, the critical processes in cuticle development could take place early in
329 grain development and involve the synthesis of cuticular material. The identification of a
330 particular chemical pathway responsible for the phenomenon of skinning is ongoing.

331

332 The use of enzymes as hull loosening treatments indicated that hulls were not as susceptible
333 to pectinase in the same way that endosperm cell walls were. It has long been established that
334 the presence of a pectin-rich layer along a cuticle and cell wall interface eases cuticle
335 separation by enzymatic hydrolysis (Norris and Bukovac, 1968). Cuticular membranes have
336 been isolated from the leaves of many species using pectinase, but in some cases this can
337 only be achieved before the leaves are fully developed (Jeffree, 1996). Furthermore,
338 structural and chemical variation in some species impedes the process of cuticle enzymatic
339 isolation (Guzman et al., 2014). The failure of the hull to detach from the caryopsis after
340 treatment with pectinase may indicate that no pectin lamella forms outside the secondary cell
341 wall of the pericarp epidermis. Alternatively, it is also possible that pectinaceous material is
342 deposited under the cuticle proper of the pericarp epidermis very early in grain development
343 but that as the cuticular membrane matures, the pectinaceous material is enveloped in cutin. It
344 thus becomes unavailable either to enzymes in aqueous solutions (Jeffree, 1996).

345

346 The severity of grain skinning in barley is known to be influenced by the environment as well
347 as harvesting and handling methods (Aidun et al., 1990; Olkku et al., 2005; Psota et al., 2011;
348 Roumeliotis et al., 2001). Mechanical impact is required to cause hull loss, but different
349 varieties have different susceptibilities to skinning (Olkku et al., 2005). Crosses involving
350 Harrington, a cultivar prone to skinning, indicated that heritability of skinning was relatively
351 low and that much of the variability observed in this trait was due to environmental factors
352 (Aidun et al., 1990). Although there is very little scientific literature on the causes of grain
353 skinning in barley, anecdotal evidence from field observations and malting the industry in the
354 UK and Germany suggest that some weather patterns (e.g. changes in atmospheric humidity
355 or intermittent wet and dry weather) may increase the risk of skinning. This is supported by
356 the work of Hamachi et al. (1989, 1990) who observed that the growth of the lemma and
357 palea in malting barley was strongly affected by environmental conditions, with poor hull
358 development linked to shading or low temperature combined with excess soil moisture.

359

360 Environmental and genetic influences on the quality of hull adhesion can be inferred from
361 more general information known about plant surface cuticles (Richardson et al., 2007;
362 Shepherd and Griffiths, 2006). The amount and composition of cuticular material synthesised
363 is influenced by environmental conditions as light, temperature and humidity or plant stress
364 and differs not only from species to species but also from organ to organ in any one plant
365 (Kolattukudy, 1996; Shepherd and Griffiths, 2006). In surface lipid extracts of naked and
366 covered barley caryopses, a lipid was shown to be present in covered barley that was not
367 present in naked barley (Kakeda et al., 2011) providing evidence that the composition of the
368 lipid is influential on the quality of hull adhesion. More directly, barley cultivars with lower
369 proportions of acetone extractives from the hull tended to exhibit poor hull-caryopsis
370 adhesion (Olkku et al., 2005). Evidence to date indicates that the genetic control of grain

371 skinning is distinct from the major gene that controls the difference between covered and
372 naked (hulless) barley lines as reported by Taketa et al. (2008). No skinning quantitative trait
373 loci have been identified in the region of the naked gene locus on chromosome 7H
374 (Rajasekaran et al., 2004), supporting the view that genotypes of cultivars exhibiting skinning
375 are different from that of naked barley. Expression of the barley *Nud* gene in transgenic rice
376 did not result in hull-caryopsis adhesion, with a minor change in lipid composition in only
377 some transgenic lines (Kakeda et al., 2011). These authors speculated that the timing of *Nud*
378 expression could be a critical factor in achieving hull adhesion. We propose that variations in
379 epicuticular wax biosynthesis and/or grain development are likely to affect the amount and/or
380 form of wax deposited between the lemma/palea and pericarp. Coupled with the amount of
381 grain fill this will affect the degree of adhesion between the hull and pericarp. In grain
382 exhibiting skinning the quality of the cementing material is apparently compromised and
383 partial or complete hull loss is observed.

384

385 **5. Conclusions**

386 The critical developmental processes necessary for good quality hull-caryopsis adhesion
387 appear to take place early in grain development, and to be mediated through the lipid
388 cementing layer. The critical adhesion phase occurs when the grain is between early to soft
389 dough. Grain skinning is influenced by a number of developmental mechanisms that relate to
390 both grain growth and the quality of the cementing layer. Therefore, expression of genes
391 underlying mechanisms that control grain skinning is likely to be under considerable
392 environmental influence. The findings reported herein provide a basis for ongoing research
393 into phenotypic expression among barley cultivars and the identification of the physiological
394 and genetic controls of the hull adhesion process.

395

396 **Acknowledgements**

397 This research was supported by funding from the Agriculture and Horticulture Development
398 Board (AHDB), the Scottish Government's Rural and Environment Science and Analytical
399 Services Division (RESAS) and the BBSRC Crop Improvement Research Club (CIRC).

400

401 **References**

402 Aidun, V.L., Harvey, B.L., Rossnagel, B.G., 1990. Heritability and genetic advance of hull
403 peeling in two-row barley. *Can. J. Plant Sci.* 70, 481-485.

404

405 Agu, R.C., Devenny, D.L., Tillett, I.J.L., Palmer, G.H., 2002. Malting performance of normal
406 hullless and acid dehulled barley samples. *J. Inst. Brew.* 108(2), 215-220.

407

408 Agu, R.C., Bringhurst, T.A., Brosnan, J.M., 2008. Performance of hulled, acid dehulled and
409 hull-less barley and malt in relation to alcohol production. *J. Inst. Brew.* 114(1), 62-68.

410

411 Bryce, J.H., Goodfellow, V., Agu, R.C., Brosnan, J.M., Bringhurst, T.A., Jack, F.A., 2010.
412 Effect of different steeping conditions on endosperm modification and quality of distilling
413 malt. *J. Inst. Brew.* 116(2), 125-133.

414

415 Brundrett, M.C., Kendrick, B., Peterson, C.A., 1991. Efficient lipid staining in plant material
416 with Sudan Red 7B or Fluoral Yellow 088 in polyethylene glycol-glycerol. *Biotechnic and*
417 *Histochem.* 66, 111-116.

418

419 Cochrane, M.P., 1985. Assimilate uptake and water loss in maturing barley grains. *J. Exp.*
420 *Bot.* 36, 770-782.

421

422 Cochrane, M.P., 1994. Observations on the germ aluerone of barley. Morphology and
423 histochemistry. *Ann. Bot.* 73, 113-119.

424

425 Cochrane, M.P., Duffus, C.M., 1979. Morphology and ultrastructure of immature cereal
426 grains in relation to transport. *Ann Bot.* 44, 67-72.

427

428 Cochrane, M.P., Paterson, L., Gould, E., 2000. Changes in chalazal cell walls and in the
429 peroxidase enzymes of the crease region during grain development in barley. *J. Exp. Bot.* 51,
430 507-520.

431

432 Dupont, F.M., Altenbach, S.B., 2003. Molecular and biochemical impacts of environmental
433 factors on wheat grain development and protein synthesis. *J. Cereal Sci.* 38, 133-146.

434

435 Freeman, P.L., Palmer, G.H., 1984. The structure of the pericarp and testa of barley. *J. Inst.*
436 *Brew.* 90, 88-94.

437

438 Gaines, R.L., Bechtel, D.B., Pomeranz, Y., 1985. A microscopic study on the development
439 of a layer in barley that causes hull-caryopsis adherence. *Cereal Chem.* 62, 35-40.

440

441 Guzmán, P., Fernández V.G., García, M.L., Khayet, M., Fernández, A., Gil, L., 2014.
442 Localization of polysaccharides in isolated and intact cuticles of eucalypt, poplar and pear
443 leaves by enzyme-gold labelling. *Plant Physiol. Bioch.* 76, 1-6.

444

445 Hamachi, Y., Furusho, M., Yoshida, T., 1989. Hull development and the cause of
446 underdevelopment of hulls in malting barley. *Japan. J. Crop Sci.* 58, 507-512.
447

448 Hamachi, Y., Yoshino, M., Furusho, M., Yoshida, T., 1990. Hull size and underdevelopment
449 of hulls under excess soil moisture condition in malting barley. *Japan. J. Crop Sci.* 59,
450 667-671.
451

452 Harlan, H.V., 1920. Daily development of kernels of Hannchen barley from flowering to
453 maturity, at Aberdeen Idaho. *J. Agric. Res.* 19, 393-429.
454

455 Jeffree, C.E., 1996. Structure and ontogeny of plant cuticles. In: Kerstiens, G. (Ed.), *Plant*
456 *Cuticles*. Oxford: Bios Scientific Publishers Limited, pp. 33-82.
457

458 Kakeda, K., Ishihara, N., Izumi, Y., Sato, K., Taketa, Shin., 2011. Expression and functional
459 analysis of the barley Nud gene using transgenic rice. *Breeding Sci.* 61, 35-42.
460

461 Kolattukudy, P.E., 1996. Biosynthetic pathways of cutin and waxes, and their sensitivity to
462 environmental stresses. In: Kerstiens, G. (Ed.), *Plant Cuticles*. Oxford: Bios Scientific
463 Publishers Limited, pp. 83-108.
464

465 Norris, F., Bukovac M.J., 1968. Structure of the pear leaf cuticle with special reference to
466 cuticular penetration. *Am. J. Bot.* 55, 975-983.
467

468 Olkku, J., Kotaviita, E., Salmenkalli-Marttila, M., Sweins, H., Home, S., 2005. Connection
469 between structure and quality of barley hull. *J. Am. Soc. Brew. Chem.* 63, 17-22.

470

471 Psota, V., Lukšičková, E., Ehrenbergerová, J., Hartmann, J., 2011. The effect of the genotype
472 and environment on damage of barley grains (*Hordeum vulgare* L.) Cereal Res. Comm. 39,
473 246-256.

474

475 Rajasekaran, P., Thomas, W.T.B., Wilson, A., Lawrence, P., Young, G., Ellis, R.P., 2004.
476 Genetic control over grain damage in a spring barley mapping population. Plant Breed. 123,
477 17-23.

478

479 Richardson, A., Boscari, A., Schreiber, L., Kerstiens, G., Jarvis, M., Herzyk, P., Frickeet,
480 W., 2007. Cloning and expression analysis of candidate genes involved in wax deposition
481 along the growing barley (*Hordeum vulgare*) leaf. Planta 226, 1459-1473.

482

483 Roumeliotis, S., Collins, H.M, Logue, S.J., Willsmore, K.L., Jefferies, S.P., Barr, A.R., 1999.
484 Implications of thin hull in barley. Australian Barley Technical Symposium. The University
485 of Adelaide, Australia. <http://www.regional.org.au/au/abts/1999/roumeliotis.htm?print=1>

486

487 Roumeliotis, S., Logue, S.J., Hunt, C., Barr, A.R., 2001. Pre-release characterisation of the
488 malting profile of WI-3102. <http://regional.org.au/au/abts/2001/t4/roumelioti.htm>

489

490 Scott, W.R., Appleyard M., Fellowes, G., Kirby, E.J.M., 1983. Effect of genotype and
491 position of ear on carpel and grain growth and grain weight of spring barley. J Agric. Sci.
492 100, 383-391.

493

494 Shepherd, T., Griffiths, D.W., 2006. The effects of stress on plant cuticular waxes. Tansley
495 Review. *New Phytol.* 171, 469-499.

496

497 Taketa, S., Amano, S., Tsujino, Y., Sato, T., Saisho, D., Kakeda, K., Nomura, M., Suzuki, T.,
498 Matsumoto, T., Sato, K., Kanamori, H., Kawasaki, S., Takeda, K., 2008. Barley grain with
499 adhering hulls is controlled by an ERF family transcription factor gene regulating a lipid
500 biosynthesis pathway. *Proc. Natl. Acad. Sci. USA* 105, 4062-4067.

501

502 Tottman, D.R., Broad, H., 1987. The decimal code for the growth stages of cereals, with
503 illustrations. *Ann. Appl. Biol.* 110, 441-454.

1 **Table 1**

2 Stages of grain development in days after anthesis and according to growth stages and
3 decimal code as described by Tottman and Broad (1987).

Days after anthesis	Growth stage	Decimal code
4	Watery ripe	GS71
10	Medium milk	GS75
14	Late milk	GS77
19	Early dough	GS83
27	Soft dough ^a	GS85
44	Hard dough ^a	GS87

4

5 ^a Assessment of the transition from soft to hard dough can be subjective and grains
6 harvested at 37 or 38 daa were intermediate to GS85 and GS87.

Fig. 1. Caryopsis growth and development at days after anthesis. a) Volume (open symbols) and moisture content (closed symbols); b) fresh weight (open symbols) and dry weight (closed symbols); c) length (open symbols) and width (closed symbols). Data points are mean values with standard error bars. The hull-caryopsis adhesion phase is indicated from 18 to 27 daa.

Fig. 2. Lemma and palea development at days after anthesis. a) length; b) width and c) dry weight for lemma (open symbols) and palea (closed symbols). Data points are mean values with standard error bars. The hull-caryopsis adhesion phase is indicated from 18 to 27 daa.

Fig. 3. Light microscopy of hull to caryopsis adhesion. a) The hull (lemma) is in contact with the underlying pericarp at the dorsal area of a grain i.e. the grain has not skinned. The pericarp epidermal cells are compressed (arrow). A vascular bundle is present within the hull, in mid-picture. The bar is 100 μm . b) The lemma is losing contact with the underlying pericarp at the dorsal area of a grain (arrow) i.e. the grain is skinning. The bar is 200 μm . c) Gape between the lemma (left) and palea (right) overlying the pericarp that encloses the aleurone layer (block-like cells) and the starchy endosperm within. The bar is 200 μm . d) Fine edge of the lemma in a gaping grain. The lemma overlies the compressed cells of the pericarp and the block-like cells of the aleurone layer. The bar is 100 μm . For a), b) and c) grains were at 24 daa, and in d) the grain was at 45 daa.

Fig. 4. Microscopy of transverse sections of barley grains stained with Fluorol Yellow. a) On the dorsal side of a grain, the inner surface of the lemma (Le) is bounded by the cementing layer (cl). The pericarp overlies under the testa cuticular layer (arrowed, tcl) and nucellus cuticular layer (arrowed, ncl) and the aleurone (Al). The grain was harvested 38 daa. The bar is 200 μm . b) On the ventral side of a grain, the cementing layer (cl) is between the palea (Pa) and pericarp (Pe). The testa cuticular layer (tcl) is evident along the inner surface of the pericarp. This section is from the chalazal region of the grain (Ch) and at 45 daa. The bar is 100 μm .

Fig. 5. Transmission electron micrographs from transverse sections cut from the middle of barley grains. a) Lignified walls (Lw) of the cells of the outer epidermis of the lemma overlay the pericarp (Pe). The cementing layer between the lemma and pericarp (long arrow), testa cuticular membrane (short arrow) and nucellus cuticular membrane (arrow head) are shown, as is the outer part of the aleurone (Al). The bar is 10 μm . b) The lemma (Le) is attached to the pericarp (Pe). An electron dense line is evident in the cementing layer (long arrow) and a bubbly boundary, thought to be globular deposits of cutin (arrow head), is present along the outer surface of the pericarp. The bar is 500nm. c) Further evidence for an electron dense layer (arrow) and a bubbly layer (bu) along the outer side of the pericarp. The bar is 1 μm . d) The inner surface of the pericarp (Pe) is bounded to the testa cuticular membrane (tcm) with its cuticle proper (arrowed). A reticulate component (rc) in the tcm is evident. The nucellus cuticular membrane (ncm) lies between the testa (Te) and nucellus (Nu). The outer part of the aleurone (Al) is shown. Bar represents 1 μm . e) The cementing layer between the lemma (Le) and pericarp (Pe) has an electron dense layer (arrows), with evidence of a split in this layer (left side). Bar represents 500nm. For a), b), c) and d) grains were at 45 daa, and in e) the grain was at 24 daa.

Fig. 1. Caryopsis

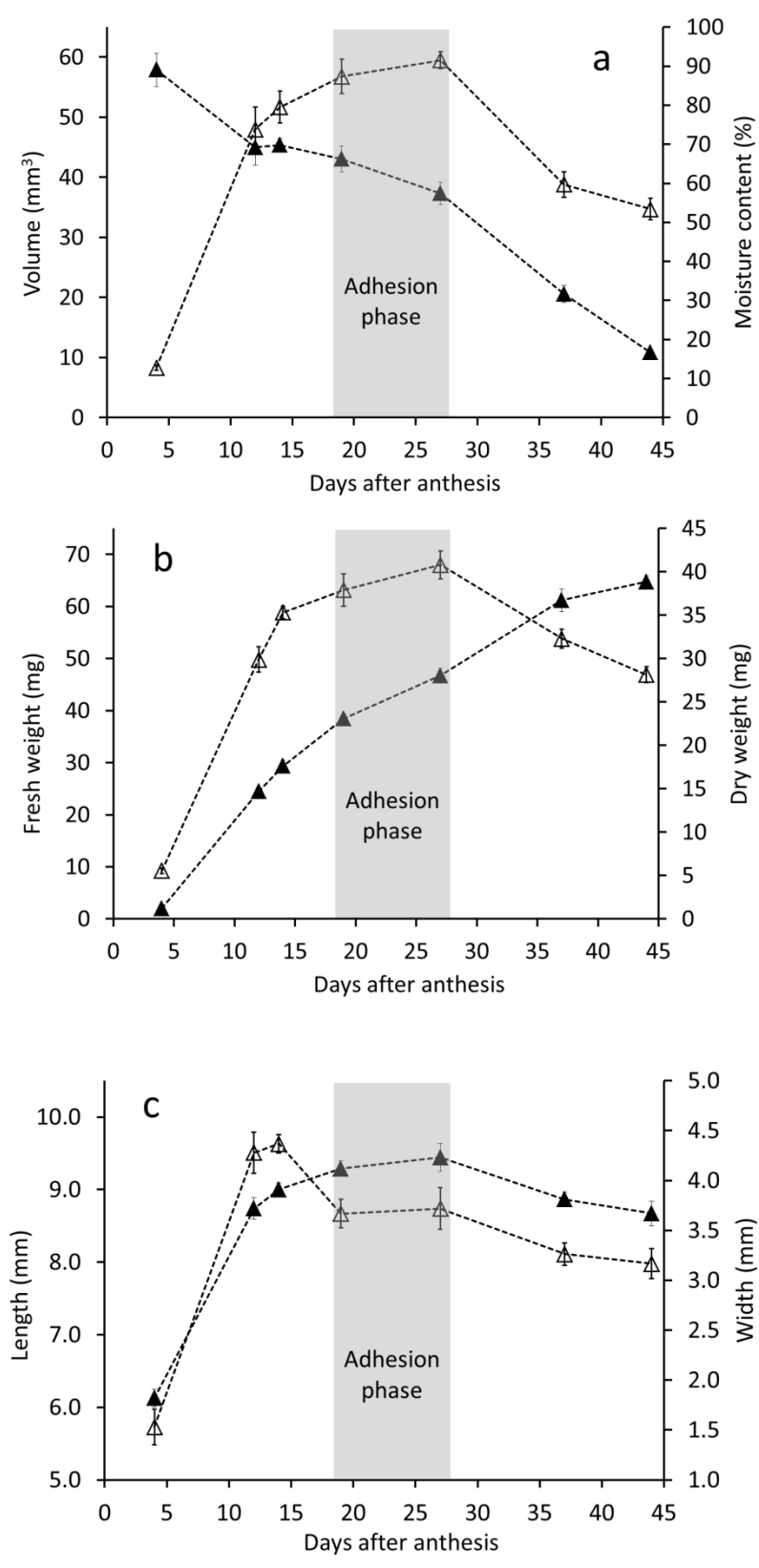


Fig. 2. Lemma and palea

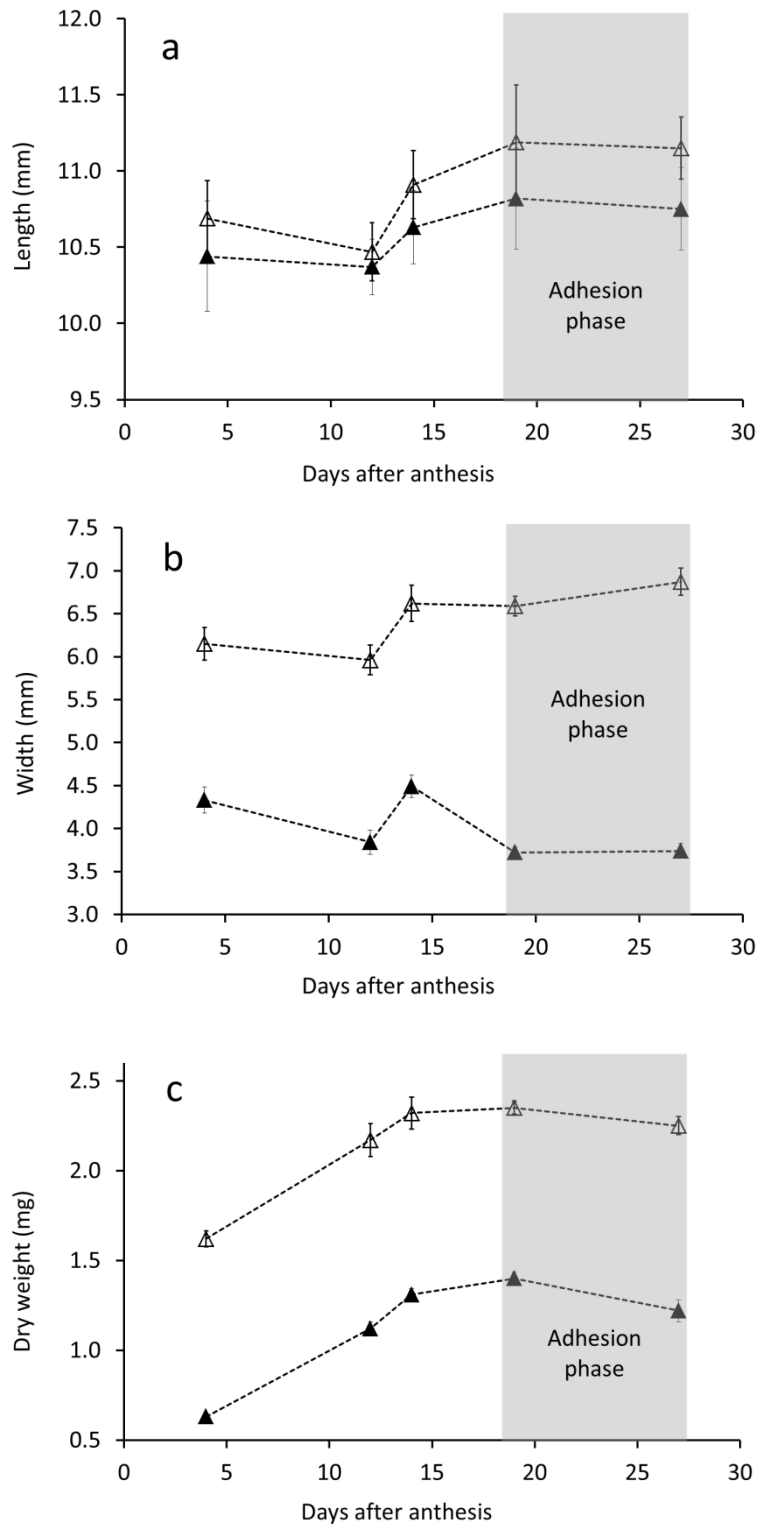


Fig. 3. Light microscopy

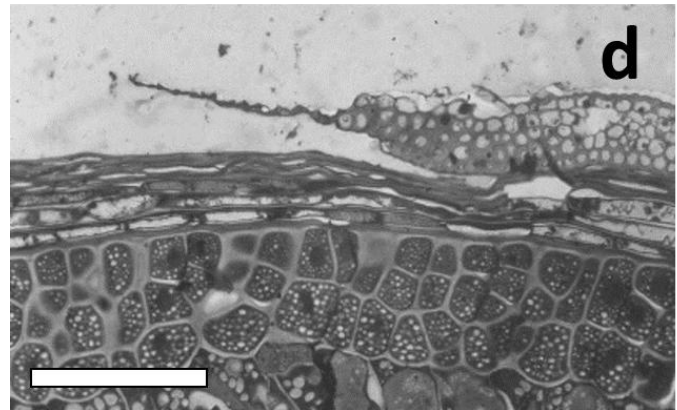
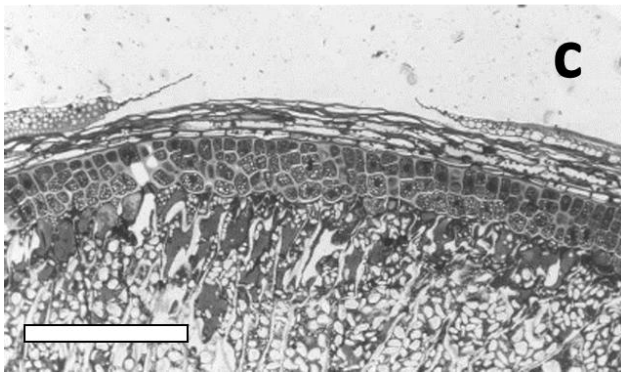
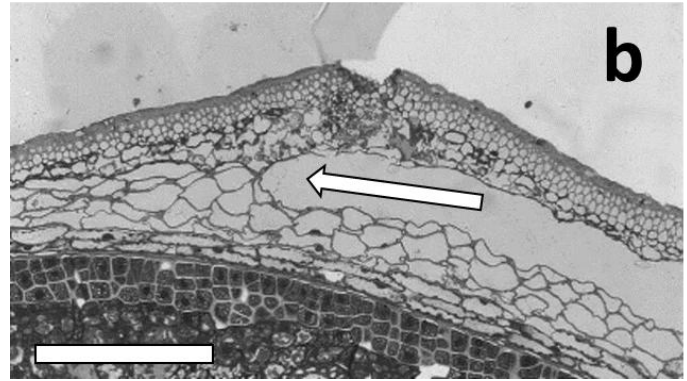
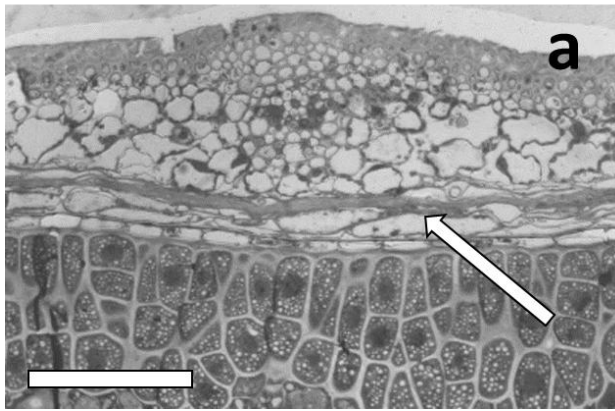


Fig. 4. Fluorescence microscopy

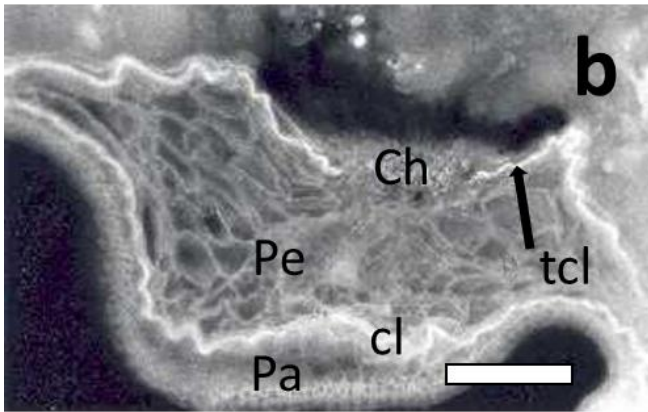
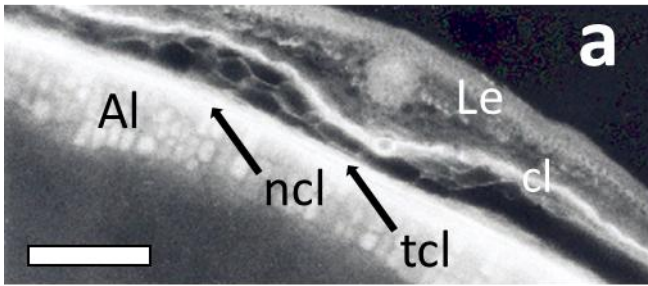


Fig. 5. TEM

