Ann. Bot. **51**, 119–130, 1983

A Quantitative Scale of Spike Initial and Pistil Development in Barley and Wheat

S. R. WADDINGTON, P. M. CARTWRIGHT

Department of Agricultural Botany, University of Reading, Berkshire, RG6 2AS

and P. C. WALL

CIMMYT, Londres 40, Apdo. Postal 6–641, México 6, D.F. México

Accepted 1 June 1982

ABSTRACT

Spring barley cv. Koru and spring wheat cv. Highbury were grown in constant controlled conditions of 16 h photoperiod, and temperatures of 10 and 15 °C respectively. A range of spike growth and developmental attributes were closely monitored from seedling emergence to pollination in the most advanced floret.

Simple, quantitative scales of development from seedling emergence (0) to pollination (10) are proposed, based on the morphogenesis of the spike initial, then the floret and finally the pistil. These scales allow developmental (ontogenetic) progress to be quantified without involving any attribute of growth or size of the plant or its organs.

Key words: *Hordeum sativum* Jess., *Triticum aestivum* L., barley, wheat, quantification of development, ontogenesis, morphogenesis, growth, spike initial, pistil, gynoecium.

INTRODUCTION

In both barley and wheat the determination of grain number during the pre-heading phase frequently appears to be the major constraint to grain yield. During this period aspects of spike development (morphogenesis of floral parts) as well as spike growth greatly influence the production and survival of florets and thus grain number. Many workers have investigated the effects of daylength and temperature on spike growth and development (e.g. Nicholls and May, 1963; Thorne, Ford and Watson, 1968; Warrington, Dunstone and Green, 1977; Kirby and Appleyard, 1980) while others (e.g. Fisher, 1973; Holmes, 1973; Brooking and Kirby, 1981) have concentrated on genetic effects, particularly of the Norin 10 genes in wheat. More recently, there has been considerable interest in the hormonal manipulation of growth and developmental phenomena within the spike (Williams and Cartwright, 1980; Hutley–Bull and Schwabe, 1982; Waddington and Cartwright, unpublished data). Such studies have shown that the rates of these two groups of processes can, to some degree, be manipulated independently by chemical growth regulators such as 6-benzylaminopurine, gibberellic acid, chlormequat and mepiquat chloride with effects on grain yield. For work in all of these areas to be of maximum value the precise measurement and quantification of both growth and development is essential.

Most published scales of spike morphogenesis define distinct morphological stages of spikelet development prior to pistil initiation, based on descriptions by Bonnett (1935, 1936 and 1966), Sharman (1947) and Barnard (1955). They include the scales for barley proposed by Andersen (1952), Aspinall and Paleg (1963), Nicholls and May (1963), Banerjee and Wienhues (1965), Nicholls (1974) and those for wheat by Andersen (1954), Friend, Fisher and Helson (1963), Banerjee and Wienhues (1965), Williams (1966),

0305-7364/83/010119+12 $03.00/0

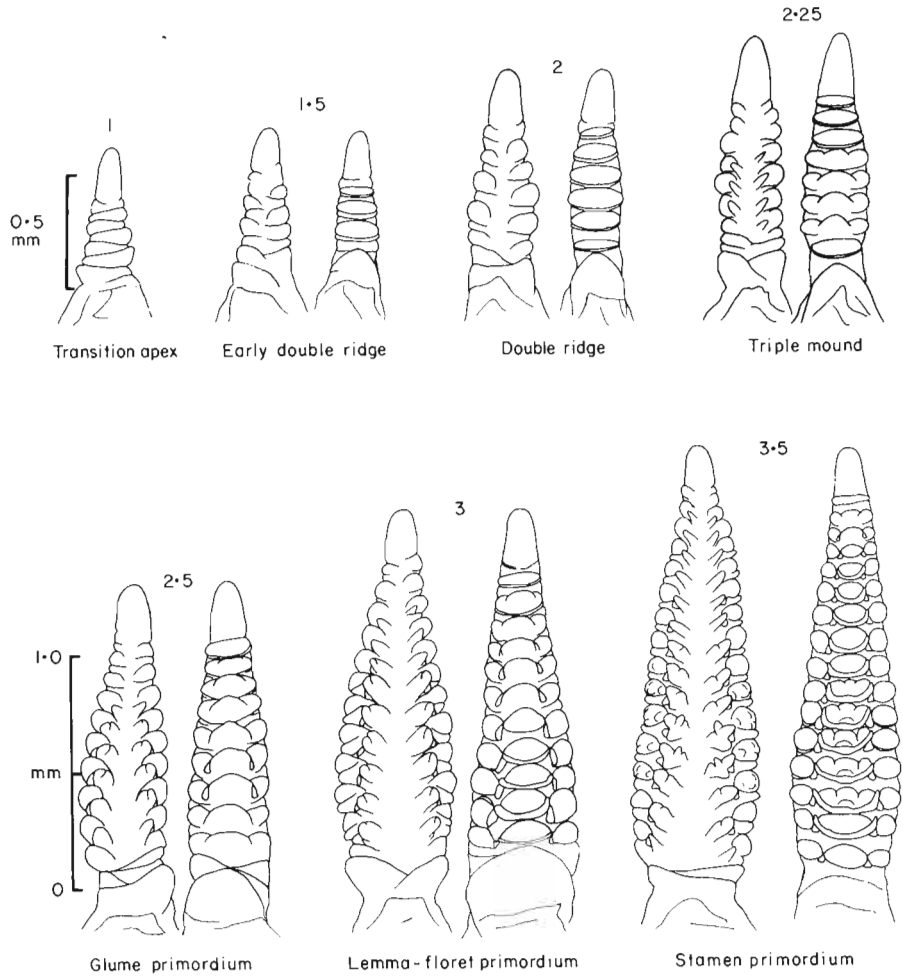

FIG. 1. Scale of development for barley. Morphogenesis of the spike initial. Numbers above the drawings indicate values on the scale (0–10).

Nicholls (1974), and Nerson, Sibony and Pinthus (1980). After pistil initiation they revert to growth characters that vary greatly with cultivar, e.g. anther length, awn length and spike emergence. The Cereal Development Guide (Kirby and Appleyard, 1981) presents a recent, comprehensive description of spike development and growth but it is also based on 'growth' characters after pistil initiation. Indeed, few detailed studies of pistil morphogenesis have been reported, although Bonnett (1966) and Williams (1966) illustrated certain distinct stages and Wall (1979) distinguished 14 stages of its morphogenesis in wheat.

Of the scales referred to only those by Friend *et al.* (1963) and Williams (1966) can be regarded as quantitative. They are based on the number of days taken to reach given morphological stages under defined conditions (30 °C, 24 h photoperiod, 2500 ft-candle and Marquis wheat for Friend *et al.*, 1963 and 20/15 °C, 24 h daylength and Nabawa wheat for Williams, 1966). The scale devised by Gregory and Purvis (1938) and as extended by Bruinsma and Swart (1962) for rye was similarly based.

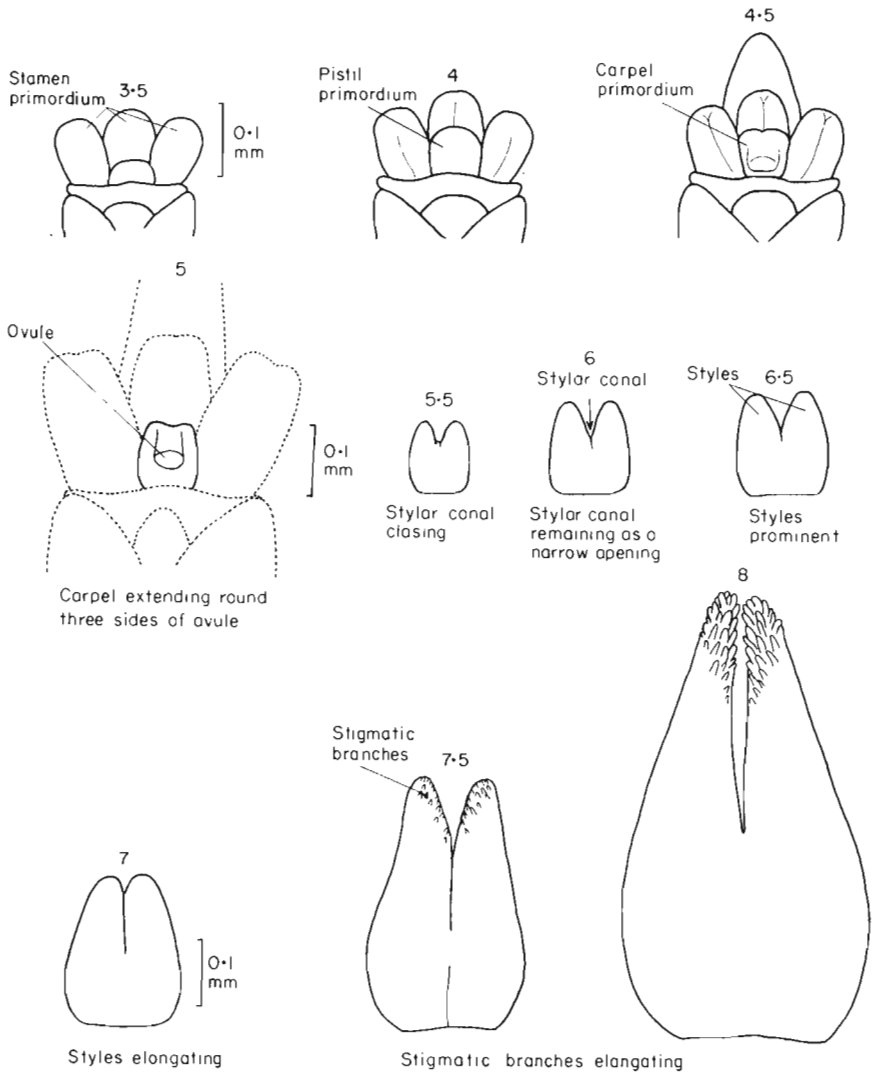

FIG. 2. Scale of development for barley. Early stages in the morphogenesis of the pistil.

In this paper we present simple, quantitative scales of development for barley and wheat, commencing at the 'transition apex' and continuing through floret and pistil (gynoecium) morphogenesis to pollination.

MATERIALS AND METHODS

The two-row spring barley, Koru and the spring wheat, Highbury were grown in pots under growth room conditions at a density of 400 plants per m^2. Temperatures were maintained at 10 °C for barley and 15 °C for wheat over a 16/8 h light/dark period with a photosynthetic (400–700 nm) photon flux density during the light period at plant height of 145 μmol s^{-1} m^{-2} provided by warm white fluorescent tubes (88 per cent on a wattage basis) and incandescent bulbs (12 per cent). Three or four plants of each species were

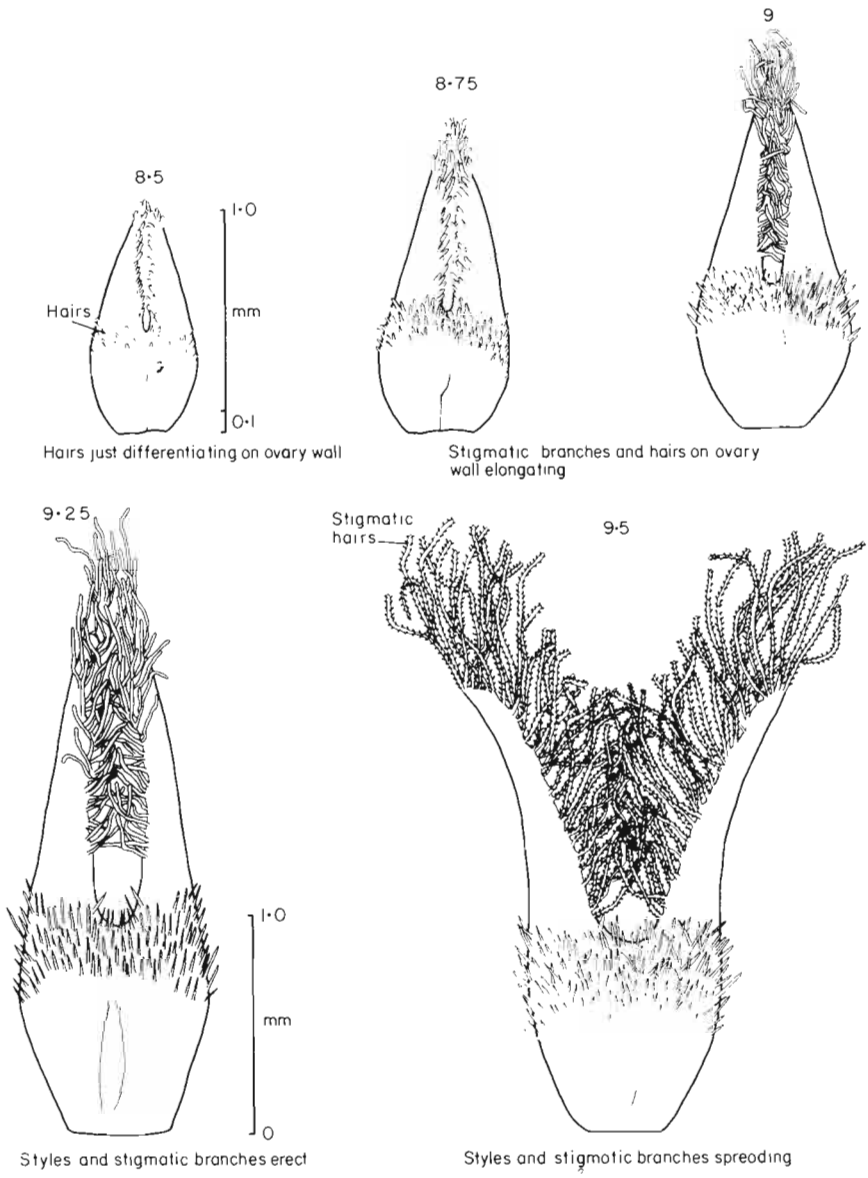

FIG. 3. Scale of development for barley. Later stages in the morphogenesis of the pistil.

examined at approximately 2-day intervals from seedling emergence to pollination of the most advanced floret. Attributes measured included spikelet number, spike length, awn length, anther length and morphological state of the most advanced floret (or pistil). Dissections were performed under a stereo microscope and the drawings of the spike initials and pistils prepared using a camera lucida. Specimens for scanning electron microscopy were fixed in 3 per cent glutaraldehyde, post-fixed in 1 per cent osmic acid, dehydrated in acetone and dried in a critical point dryer. Specimens were coated in platinum and examined in a Jeol JSM-T20 scanning electron microscope.

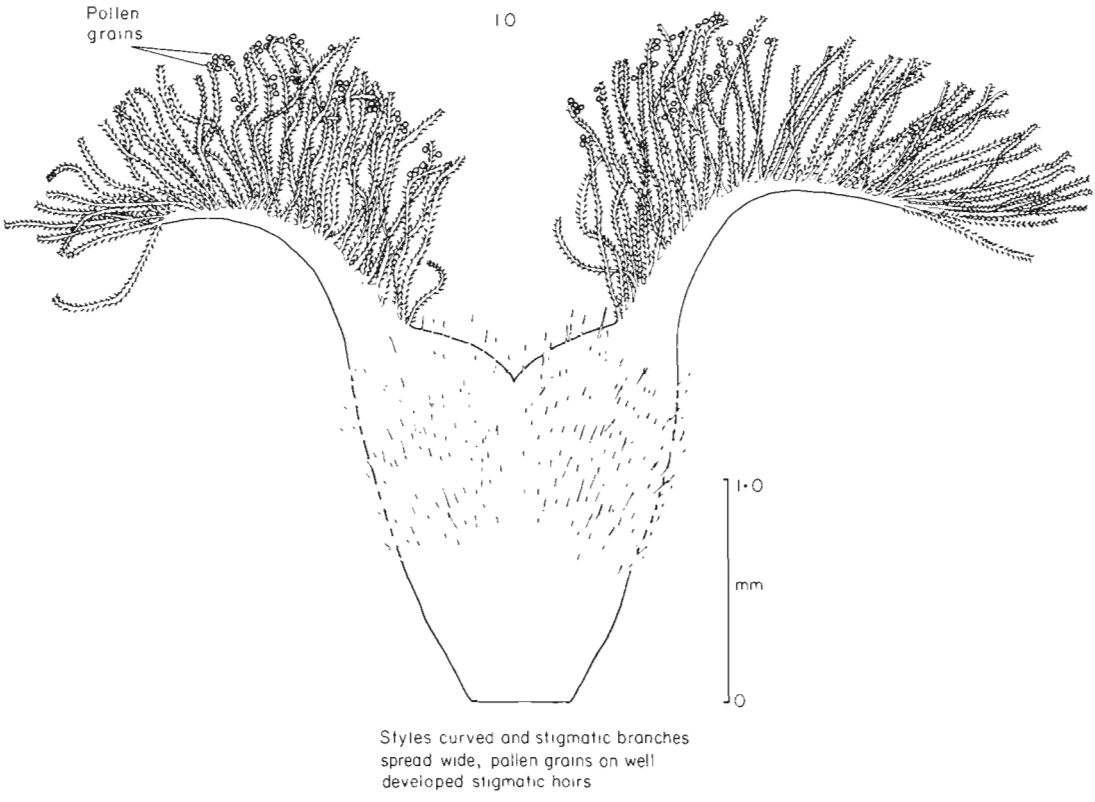

FIG. 4. Scale of development for barley. Pistil at pollination.

THE SCALES

The essential feature of a developmental process is programmed qualitative change in form. In the present context this refers to progress towards pollination, i.e. spike morphogenesis. In contrast, growth is a quantitative process, measured as an increase in biomass, length, volume, number, etc. To assess development quantitatively the programme needs to be defined as a progression through identifiable stages where each stage is equated to progress through a certain fraction of the whole programme. The proposed scales are based on regularly spaced (in terms of day degrees or time) stages of spike morphogenesis under defined conditions of daylength and temperature, easily identifiable after microdissection. Being based on pistil differentiation for the appropriate period the scales focus on the organ with the potential to become the grain. The pistil also displays a more marked, discontinuous change in morphology and growth during the pre-pollination period than do either the anthers or the lemma–awn complex.

The scale for barley (Figs 1–5) and that for wheat (Table 1 and Fig. 6) show the time (and since temperature was held constant, day °C) taken to reach a given morphological stage as a fraction of the time taken to reach pollination in the most advanced floret. The bounds of the scales are seedling emergence (vegetative apex) (0) and pollination in the most advanced floret (10). Brief descriptions of the morphological stages are also given.

Table 2 (for barley) and Table 3 (for wheat) represent an attempted comparison of

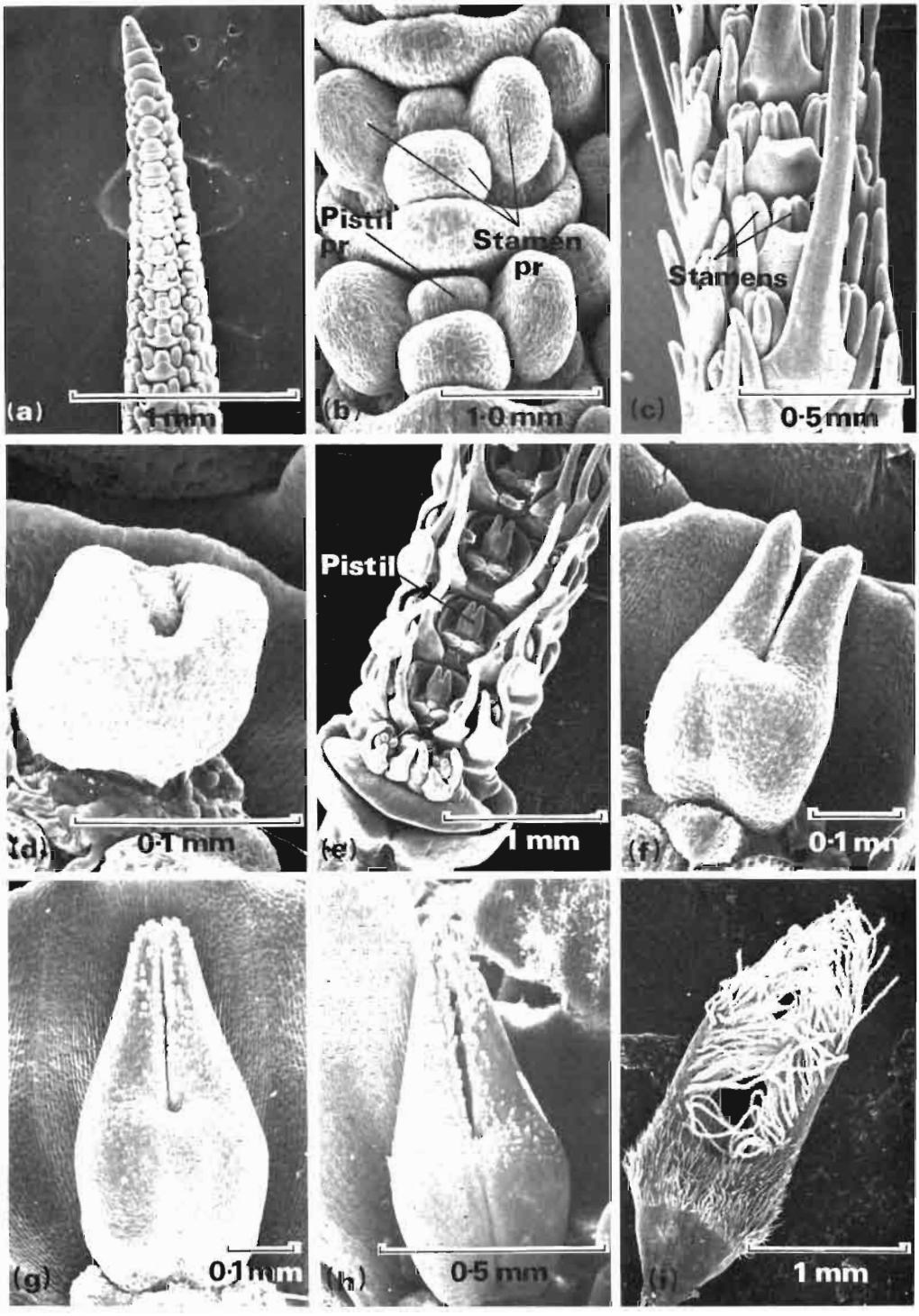
(a)
1 mm
(b)
Pistil pr
Stamen pr
1·0 mm
(c)
Stamens
0·5 mm
(d)
0·1 mm
(e)
Pistil
1 mm
(f)
0·1 mm
(g)
0·1 mm
(h)
0·5 mm
(i)
1 mm

FIG. 5

TABLE 1. *Scale of spike initial and pistil morphogenesis for wheat*

Developmental score (stage)	Description	Days after emergence
1·5	Transition apex	14
2	Early double ridge stage	20
2·5	Double ridge stage	26
3	Glume primordium present	30
(3·25)	Lemma primordium present	32
3·5	Floret primordium present	35
4	Stamen primordium present	38
(4·25)	Pistil primordium present	41
4·5	Carpel primordium present	46
5	Carpel extending round three sides of ovule	49
5·5	Stylar canal closing; ovarian cavity enclosed on all sides but still open above	53
6	Stylar canal remaining as a narrow opening; two short round style primordia present	59
6·5	Styles begin elongating [Fig. 6(a)]	64
7	Stigmatic branches just differentiating as swollen cells on styles	69
7·5	Unicellular hairs just differentiating on ovary wall; stigmatic branches elongating	72
8	Stigmatic branches and hairs on ovary wall elongating [Fig. 6(b)]	78
8·5	Stigmatic branches and hairs on ovary wall continue to elongate; stigmatic branches form a tangled mass	82
9	Styles and stigmatic branches erect; stigmatic hairs differentiating [Fig. 6(c)]	89
9·5	Styles and stigmatic branches spreading outwards. Stigmatic hairs well developed	93
10	Styles curved outwards and stigmatic branches spread wide; pollen grains on well-developed stigmatic hairs	98

some previously published scales with those proposed in this paper. At least over the later part of our scales only approximate comparisons could be made since in this period the other scales are based on 'growth' characters, not pistil morphogenesis. In Tables 2 and 3, such 'growth' values are bracketed, e.g. stage 10 on the scale of Banerjee and Wienhues (1965) which is defined as 'awns growing' for barley coincided with closure of the stylar canal, stage 5·5 on our scale. In addition, single values on the other scales were often found to correspond to a range of values on our scales (as indicated by the

FIG. 5. Scanning electron micrographs of selected stages in the morphogenesis of the spikelet and pistil in barley. (a) Part of a barley spike initial (at stage 4, the pistil primordium stage) showing acropetal succession of progressively less advanced median spikelets. (b) Detail of (a) showing two median spikelets. The pistil primordium (just differentiating) is surrounded by three, more advanced stamen primordia. The lemma is still very small and without an awn. (c) More advanced spike in a similar view to (a). Notice the long awn and lemma and prominent glumes. The awns have been removed from the two median spikelets in the centre of the photograph revealing three anthers per spikelet. (d) Pistil with the carpel extending over all sides of ovule but still open above. (Stage 5·5). (e) View of the basal part of a spike initial. Lemma and stamens have been removed in the median spikelets to reveal position of the pistil. (f) Detail of (e) showing a pistil with two prominent styles just prior to differentiation of stigmatic branches. (Stage 7). (g) Differentiation of stigmatic branches on the styles. (Stage 7·5). (h) Differentiation of hairs on ovary wall and further elongation and differentiation of stigmatic branches. (Stage 8·5). (i) Morphologically advanced pistil showing stigmatic hairs just prior to the outward spread of stigmas and styles to receive pollen from the anthers. (Stage 9·25).

TABLE 2. *Comparison of other published scales with the scale of spike initial and pistil morphogenesis for barley: see text for explanation of numbers in parentheses and vertical dotted lines*

Developmental score							
Stage	Days after emergence	Brief description	Andersen (1952)	Aspinall and Paleg (1963)	Nicholls and May (1963)	Banerjee and Wienhues (1965)	Nicholls (1974)
1	12	Transition apex	4	2	2	3	1
1·5	16	Early double ridge stage		3–4	3	4	2
2	21	Double ridge stage	5			5	3
(2·25)	26	Triple mound present	6	5	4	6a–6b	4
2·5	28	Glume primordium present	—	6	5	7	5
3	33	Lemma-floret primordium present	7	7	6	8	6–7
3·5	37	Stamen primordium present	8	8	7	9	8
4	43	Pistil primordium present				(9a)	(9)
4·5	49	Carpel primordium present	(9)	(9)	(8)	(9b)	
5	55	Carpel extending round three sides of ovule					(10)
5·5	59	Stylar canal closing				(10)	
6	66	Stylar canal remaining as a narrow opening	(10)			(11–12)	
6·5	72	Styles prominent				(13–13a–13b)	
7	78	Styles elongating					
7·5	83	Stigmatic branches just differentiating				(14)	
8	90	Stigmatic branches elongating	(11)	(10)			(11)
8·5	95	Hairs on ovary wall just differentiating			(9)		
(8·75)	98	Stigmatic branches and hairs on ovary wall elongating				(14a)	
9	101						
(9·25)	104	Styles and stigmatic branches erect	(12)				
9·5	108	Styles and stigmatic branches spreading				(15)	
10	113	Pollination	(13)	(11)		(16–17)	

TABLE 3. *Comparison of other published scales with the scale of spike initial and pistil morphogensis for wheat: see text for explanation of numbers in brackets and vertical dotted lines*

Developmental score		Andersen (1954)	Friend *et al.* (1963)	Banerjee and Wienhues (1965)	Williams (1966)	Nicholls (1974)	Nerson *et al.* (1980)	Wall (1979)
Stage	Brief description							
1·5	Transition apex	4	11	3	16	1	2	
2	Early double ridge stage		12–13	4	22	2	3	
2·5	Double ridge stage	5	14–16	5–6	24	3–4	4–5	
3	Glume primordium present	6	17	7	26	5	6	
(3·25)	Lemma primordium present	7	18	(8)	28	6	7	
3·5	Floret primordium present	—	19	8a		7		
4	Stamen primordium present	8	20	9a	30	8	8	1
(4·25)	Pistil primordium present		21	(9b)	32	(9)	(9)	2
4·5	Carpel primordium present	(9)	(22)					3
5	Carpel round three sides of ovule		(23)	(10)	(34)	(10)		
5·5	Stylar canal closing		(24)	(11)				4–5
6	Stylar canal as narrow opening	(10)		(12)	(36)			
6·5	Styles begin elongating		(25–26)	(13)	(38)			6
7	Stigmatic branches just differentiating	(11)	(28)	(14)				7–8
7·5	Hairs on ovary wall		(32)		(42)			9
8	Stigmatic branches and ovary hairs elongating	(12)	(34)	(14a)	(46)	(11)		10
8·5	Stigmatic branches form tangled mass			(15)				
9	Styles and stigmatic branches erect		(36)	(16)	(50)			11
9·5	Styles and stigmatic branches spreading	(13)		(17)				12
10	Pollination		(38)		(55)			13–14

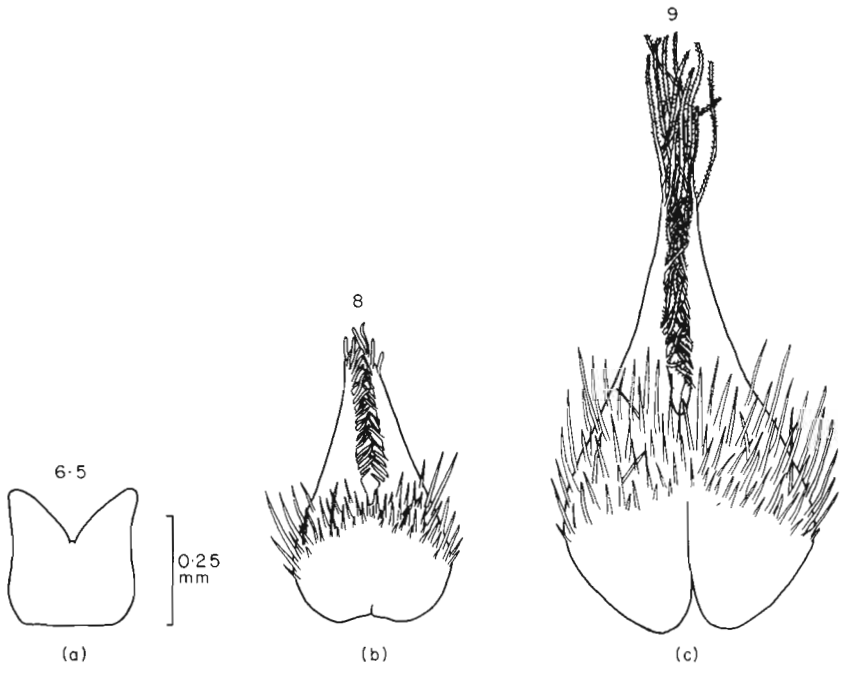

FIG. 6. Selected stages in the morphogenesis of the pistil in wheat. (a) Stage 6·5: styles begin elongating, stylar canal present as a narrow opening. (b) Stage 8: stigmatic branches and hairs on ovary wall elongating. (c) Stage 9: styles and stigmatic branches erect, stigmatic hairs differentiating.

vertical dotted lines in the tables). For instance, stage 10 on the Aspinall and Paleg (1963) scale (defined as 'awns longer than spikelets') persisted through stages 6–9·5 on our scale, i.e. for 35 per cent of the total period while their stages 6–8 were completed while our score advanced from 2·5 to 3·5, only 10 per cent of the total time.

The complex pattern of growth in a number of spike attributes and their relationship to developmental score in barley can be seen in Fig. 7. Spikelet production stops at about stage 6, shortly after initiation of the pistil while awn and anther extension do not enter an exponential phase until stage 7. Spike elongation as might have been suspected follows a more phasic growth pattern. All three length attributes show a sharp reduction in rate of growth from stage 9 to stage 10 (pollination).

The sequence of morphological events described in the scales presented occurred in all of a large range of spring and winter cereal cultivars that we have observed including Goldmarker, Ark Royal, Kym, Triumph, Igri, Sonja and Gerbel barley, and Sicco, Timmo, Cappelle and Hobbit wheat, and appears to be common to all cultivars grown in Britain at least. Although this sequence of events appears to be universal the time between developmental stages is specific to the cultivars and environmental conditions used, i.e. Koru at 10 °C and Highbury at 15 °C under 16 h daylengths. However, except where floral development is prevented by unfavourable photoperiods or inadequate vernalization, deviations from the linearity of the scales are likely to be slight. Indeed, in a contrasting environment in the field in N.W. Mexico for the semi-dwarf wheat Yecora 70, deviations were negligible. Thus, only for the most precise comparisons should it be necessary to re-calibrate the scales.

Since the proposed scales of developmental stages are independent of the stages and phases of growth they only correspond with Zadoks' or Feekes-Large 'growth stages'

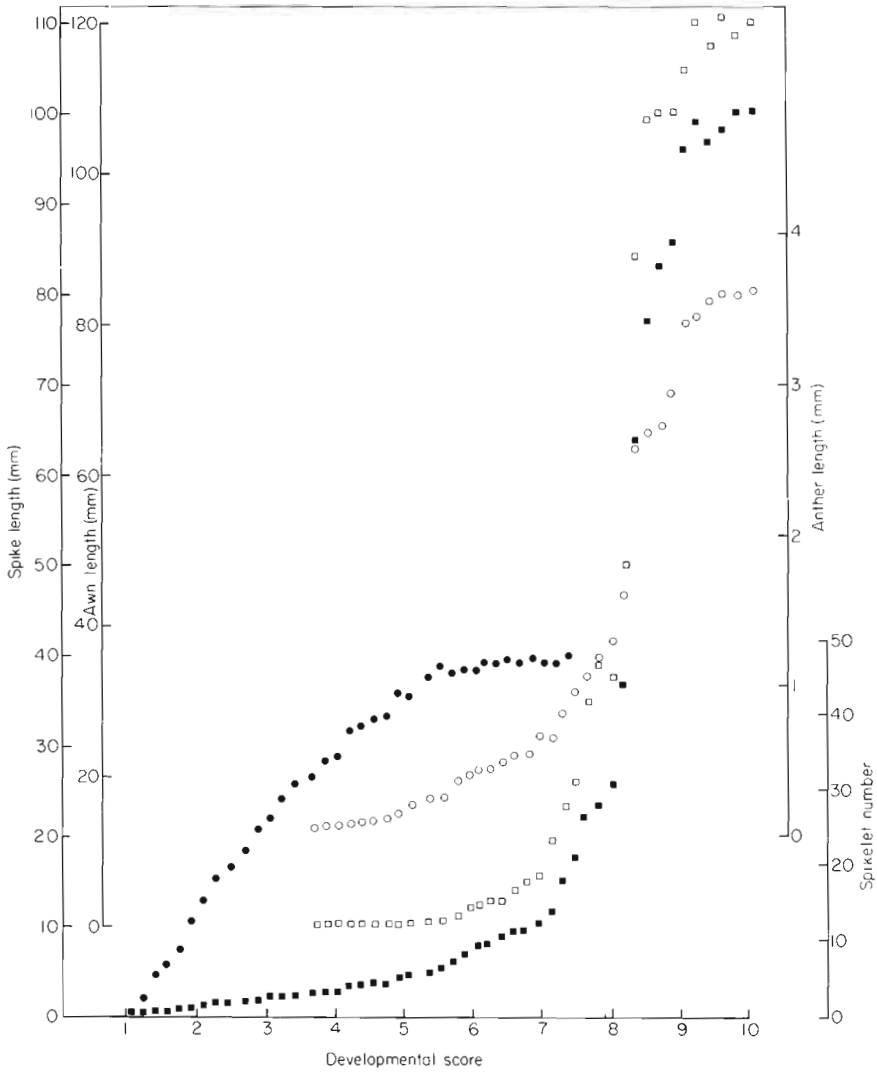

FIG. 7. Relationship between the spike growth attributes; spikelet number (●) anther length (○), spike length (■), awn length (□) and the developmental score (time after emergence) for barley.

(see Tottman, Makepeace and Broad, 1979) at points on those scales which in fact refer to related developmental features. The scales are not intended for predicting phenology under field conditions but are designed for the study of the complex relationships between the growth in size and the developmental differentiation of florets within the spike. They also provide a basis on which to compare treatments likely to affect development and growth differentially at the plant or shoot level.

ACKNOWLEDGEMENTS

This work was carried out while S.R.W. was in receipt of a Ministry of Agriculture, Fisheries and Food Postgraduate Agricultural Research Studentship. We wish to thank

Dr J. R. Barnett for his help with the scanning electron microscopy and Nickerson RPB Ltd., Rothwell, Lincoln for the supply of Koru seed.

LITERATURE CITED

ANDERSEN, S., 1952. Methods for determining stages of development in barley and oats. *Physiologia Pl.* **5**, 199–210.

—— 1954. A method for determining stages of development in wheat. Ibid. **7**, 513–6.

ASPINALL, D. and PALEG, L. G., 1963. Effects of daylength and light intensity on growth of barley. I. Growth and development of apex with a fluorescent light source. *Bot. Gaz.* **124**, 429–37.

BANERJEE, S. and WIENHUES, F., 1965. Comparative studies on the development of the spike in wheat, barley and rye. *Z. PflZücht.* **54**, 130–42.

BARNARD, C., 1955. Histogenesis of the inflorescence and flower of *Triticum aestivum* L. *Aust. J. Bot.* **3**, 1–20.

BONNETT, O. T., 1935. The development of the barley spike. *J. agr. Res.* **51**, 451–7.

—— 1936. The development of the wheat spike. Ibid. **53**, 445–51.

—— 1966. *Inflorescences of Maize, Wheat, Rye, Barley and Oats: Their Initiation and Development*. University of Illinois College of Agriculture, Agricultural Experiment Station Bulletin 721.

BROOKING, I. R. and KIRBY, E. J. M., 1981. Interrelationships between stem and ear development in winter wheat: the effects of a Norin 10 dwarfing gene, Gai/Rht_2. *J. agr. Sci., Camb.* **97**, 373–81.

BRUINSMA, J. and SWART, J., 1962. The development of the rye spike until emergence. *Acta bot. neerl.* **11**, 411–7.

FISHER, J. E., 1973. Developmental morphology of the inflorescence in hexaploid wheat cultivars with and without the cultivar Norin 10 in their ancestry. *Can. J. Pl. Sci.* **53**, 7–15.

FRIEND, D. J. C., FISHER, J. E. and HELSON, V. A., 1963. The effect of light intensity and temperature on floral initiation and inflorescence development of Marquis wheat. *Can. J. Bot.* **41**, 1663–74.

GREGORY, F. G. and PURVIS, O. N., 1938. Studies in vernalisation of cereals. II. The vernalisation of excised mature embryos, and of developing ears. *Ann. Bot.* **2**, 237–51.

HOLMES, D. P., 1973. Inflorescence development of semidwarf and standard height wheat cultivars in different photoperiod and nitrogen treatments. *Can. J. Bot.* **51**, 941–56.

HUTLEY-BULL, P. D. and SCHWABE, W. W., 1982. Some effects of low concentration gibberellic acid application at different times during growth on morphogenesis in wheat. In *Chemical Manipulation of Crop Growth and Development*, 33rd Easter School in Agricultural Science, University of Nottingham, ed. J. S. McLaren. Butterworths, London. (In Press).

KIRBY, E. J. M. and APPLEYARD, M., 1980. Effects of photoperiod on the relation between development and yield per plant of a range of spring barley varieties. *Z. PflZücht.* **85**, 226–39.

—— 1981. *Cereal Developmental Guide*, 80 pp. RASE Cereal Unit, National Agricultural Centre, Stoneleigh, England.

NERSON, H., SIBONY, M. and PINTHUS, M. J., 1980. A scale for the assessment of the developmental stages of the wheat (*Triticum aestivum* L.) spike. *Ann. Bot.* **45**, 203–4.

NICHOLLS, P. B., 1974. Interrelationship between meristematic regions of developing inflorescences of four cereal species. Ibid. **38**, 827–37.

—— and MAY, L. H., 1963. Studies on the growth of the barley apex. I. Interrelationships between primordium formation, apex length, and spikelet development. *Aust. J. biol. Sci.* **16**, 561–71.

SHARMAN, B. C., 1947. The biology and developmental morphology of the shoot apex in the Gramineae. *New Phytol.* **46**, 20–34.

THORNE, G. N., FORD, M. A. and WATSON, D. J., 1968. Growth, development and yield of spring wheat in artificial climates. *Ann. Bot.* **32**, 425–46.

TOTTMAN, D. R., MAKEPEACE, R. J. and BROAD, H., 1979. An explanation of the decimal code for the growth stages of cereals, with illustrations. *Ann. appl. Biol.* **93**, 221–34.

WALL, P. C., 1979. An analysis of factors limiting grain numbers and yield of spring wheat in a low-latitude environment. Ph.D. thesis, University of Reading.

WARRINGTON, I. J., DUNSTONE, R. L. and GREEN, L. M., 1977. Temperature effects at three development stages on the yield of the wheat ear. *Aust. J. agr. Res.* **28**, 11–27.

WILLIAMS, R. F., 1966. The physiology of growth in the wheat plant. III. Growth of the primary shoot and inflorescence. *Aust. J. biol. Sci.* **19**, 949–66.

WILLIAMS, R. H. and CARTWRIGHT, P. M., 1980. The effect of applications of a synthetic cytokinin on shoot dominance and grain yield in spring barley. *Ann. Bot.* **46**, 445–52.