GENETICAL ASPECTS OF STRAWBERRY JUNE YELLOWS

A. B. WILLS

Genetics Department, Scottish Horticultural Research Institute

Received 14.xii.61

1. INTRODUCTION

JUNE YELLOWS is a progressive, deleterious variegation of the cultivated strawberry. Repeated attempts have failed to transmit the disease, by possible insect vectors or by grafting, to unaffected plants. Heritability was first demonstrated by Clark (1930) and later by other workers, but early attempts at genetic analysis were inconclusive. Darlington and Mather (1949) suggested that the disease might be caused by a mutant plasmagene and the subsequent experimental results of Williams (1955) and McWhirter (1955) were consistent with this view.

Genetical investigation of June Yellows at this Institute was stimulated by the rapid decline of the variety Auchincruive Climax (hereinafter referred to as Climax), and the widespread occurrence of the disease in seedlings and advanced selections bred here.

2. SYMPTOMS

Symptoms appear in Scotland with the start of spring growth, reach a peak in May, and fade and disappear in late spring or early summer. They may reappear in autumn, but never at the earlier frequency or intensity.

Symptoms were described by Williams (1955) and by others. Leaves expanding in early spring vary from a nearly uniform pale yellow colour to a light mottle of green and yellow. Successively later unfolding leaves have a more conspicuous mottle, with an increase in chlorophyll colour in some cells. These stages are seen in plate 1*a*. Chlorophyll continues to increase as leaves age and the mottling may disappear. Reid (1956) reported that the development of green colour in leaves was accelerated by growing plants in a warm greenhouse. McWhirter (1955) noted that symptom distribution reflected an influence of cell lineage. Permanent white streaks, often coupled with leaf distortion, appear spasmodically in Climax, Cambridge Favourite and some other affected varieties. Symptoms vary slightly between varieties, but the segmental pattern and more or less abrupt boundaries between areas of different colour distinguish June Yellows from any other generalised chloroses that show seasonal variations.

The disease increased in intensity each year in affected plants of Climax. Badly affected plants were dwarfed, bore a much reduced

fruit crop, and eventually died. Plate 1*b* shows extreme differences in vigour between two affected sub-clones of Climax. Following the first report by Reid (1951) of June Yellows in this variety, all plants became so badly affected that by 1955 nearly all stocks were commercially valueless. Many less prominent varieties appear to have declined as rapidly in the U.S.A., while the successful selection of relatively stable green sub-clones has seldom been achieved.

3. MATERIALS AND METHODS

Climax and seven varieties were selfed and reciprocally crossed in all possible combinations. The varieties used and their June Yellows status are listed in table 1.

Parent plants were grown outside in pots until shortly prior to flowering, when they were brought into a warm (50°-70° F.) greenhouse for pollination.

Seeds were sown in boxes in November, overwintered in a gauze-covered cold

TABLE 1
Parental varieties: June Yellows status and breeding behaviour

Variety	June Yellows status	
	in clone	in seedlings
Auchincruive Climax .	Affected	Affected
Madame Moutôt .	Affected	Affected
Tardive de Leopold .	Green	Affected seedlings reported
Royal Sovereign .	Green	Affected seedlings reported
Perle de Prague .	Green	Affected seedlings reported
Huxley .	Green	No affected seedlings reported
Early Cambridge .	Green	No affected seedlings reported
Talisman .	Green	Unknown

frame, and removed to a warm greenhouse when germination began in spring. Seedlings were pricked into boxes at the one or two leaf stage and in August were planted in the field.

4. OBSERVATIONS

(i) *June Yellows in selfed progenies*

Symptoms were observed in progenies of all selfed plants of affected varieties, even where the disease was not apparent in some individual parent plants.

(a) *Climax selfed.* The proportion of affected seedlings in all families is given in table 2*a* as the cumulative percentage of the total number of seedlings planted in each year. Green seedlings were initially present in families planted in 1956, and these showed a high likelihood of becoming affected in subsequent years. No green seedlings were found in the 1957 planting. By contrast only a low proportion of affected seedlings was present in the 1958 planting; the parent plant of this family was selected from a sub-clone which had remained green up to this time. Differences in proportions of

affected seedlings from green and affected plants of Blakemore and Howard 17 were observed similarly by Williams (1955).

TABLE 2
*Cumulative percentages of affected seedlings in families
of three varieties selfed*

Year planted	Parent plant status	No. seedlings	Percentage of seedlings affected and year observed			
			1957	1958	1959	1960
(a) <i>Auchincruive Climax selfed</i>						
1956	All affected .	820	87	98	98.4	98.5
1957	All affected .	174	...	100	100	100
1958	Green . .	13	...	...	8	8
(b) <i>Mme. Moutôt selfed</i>						
1956	Green . .	24	33	63	67	67
1957	Green . .	510	...	73	77	79
1957	Affected . .	191	...	93	94	95
(c) <i>Talisman selfed</i>						
1956	Green . .	980	0.1	0.4	1.2	2.0
1957	Green . .	87	...	0	0	0

(b) Mme. Moutôt selfed. Plants of green and affected sub-clones of Mme. Moutôt were selfed. Differences between families, shown in table 2*b*, were not so extreme as for Climax-selfed. However the trends for each variety were similar, there being an annual increase in the proportions of affected seedlings in any single planting, and an increase in initial proportions in successive annual plantings.

(c) Talisman selfed. In contrast to Climax and Mme. Moutôt there exist no authentic records of June Yellows in Talisman. However, 2 per cent. of seedlings planted in 1956 became affected in four years; these were found in ten progenies totalling 476 seedlings. No affected seedlings were found in the smaller planting of 1957. The data are given in table 2*c*.

(d) Selfings of other green varieties. June Yellows was not found in seedlings of any of the other green varieties, but other types of variegation were observed, notably in seedlings of Perle de Prague. Sufficient differences in symptom expression and mode of inheritance exist to conclude that these variegations are unrelated to June Yellows.

(ii) June Yellows in inter-varietal crosses

Symptoms were found only in progenies from crosses of Climax or Mme. Moutôt and in Tardive de Leopold $\times$ Talisman. Results from the cross Climax $\times$ Perle de Prague and its reciprocal, given in table 3, illustrate the general trends in all crosses of affected plants.

TABLE 3

*Percentages of affected seedlings in Climax × Perle de Prague
and reciprocal cross families*

Year planted	Parent plant status	No. of parent plants		No. seedlings	Percentage of seedlings affected and year observed			
		Climax	Perle de Prague		1957	1958	1959	1960
Climax × Perle de Prague								
1956	Climax affected Perle de Prague green	2	1	53	17	34	38	40
1957	„	3	3	425	...	78	85	87
1958	Climax green Perle de Prague green	2	2	98	...	...	1	2
Perle de Prague × Climax								
1956	Perle de Prague green Climax affected	3	3	64	0	19	22	23
1957	„	2	2	65	...	94	94	95
1958	„	1	1	67	...	...	82	94

TABLE 4

*Green parental varieties arranged in order of decreasing June Yellows incidence
in their Climax cross progenies*

Tardive de Leopold *
Perle de Prague
Talisman
Huxley
Cambridge Early
Royal Sovereign

* Male-sterile variety.

Crossing an affected by a green variety, as compared to selfing, considerably reduced the initial proportions of affected seedlings in the resulting families, although an increase in proportions in each year of planting was noted. The annual increase of proportions of affected seedlings in plantings made in one year was also less in the crosses than in selfs.

(iii) *Inter-varietal variations in response*

In crosses between affected and green varieties the rate at which progenies became affected, and the initial incidence of the disease were modified, to varying extents, by the green parent.

The range of response is shown in text-fig. 1, where cumulative percentages of affected seedlings are plotted against the week of scoring, for four representative crosses and Climax-selfed. All families were

TEXT-FIG. 1.—Rate of June Yellows development in four intervarietal crosses and Climax-selfed. The rate was much lower in Climax $\times$ Royal Sovereign than in other progenies.

planted in 1957. The cross Tardive de Leopold $\times$ Climax showed the closest approach to the level reached in Climax-selfed progenies, while Climax $\times$ Royal Sovereign showed the least incidence of June Yellows. No consistent differences were observed between progenies from reciprocal crosses, indicating that the intervarietal variation was determined by modifying genes from the green varieties.

The green varieties used are arranged in table 4 in order of decreasing June Yellows incidence in their progeny from crosses with Climax.

TABLE 5

Percentages of affected seedlings from selfed green and affected Mme. Moutôt plants

Year planted	Parent plant status	Parent plant code no.	No. seedlings	Percentages of affected seedlings and year observed		
				1958	1959	1960
1957	Green	42	86	80	83	84
1957	"	12	106	73	74	77
1957	"	5	89	65	72	72
1957	Affected	1	71	96	97	97
1957	"	8	120	91	92	93

(iv) *Intra-varietal variations*

Results given previously have referred to summed families within a cross and consequently variation within crosses was obscured. Results from three of six families raised from green plants of Mme. Moutôt and two families from affected Mme. Moutôt are listed in table 5 to show the range of variation encountered. All families were planted in the same year. Heterogeneity analysis by the χ^2

TABLE 6
Percentages of affected seedlings from Climax $\times$ Perle de Prague

Year planted	Parent plant status		Parent plant code nos.		No. seedlings	Percentage of affected plants and year observed			
	<i>Climax</i>	<i>Perle de Prague</i>	Female	Male		1957	1958	1959	1960
1957	Affected	Green	16	18	72	1	53	60	64
"			16	18	70	6	66	73	80
			Family total .		142	4	60	66	72
1957	Affected	Green	34	25	72	11	85	94	97
"			34	25	68	10	69	81	81
			Family total .		140	11	77	88	89
1957	Severe symptoms	Green	120	56	65	23	97	100	100
"			120	56	78	44	97	99	99
			Family total .		143	35	97	99	99
			Overall totals .		425	16	78	85	87

method of Brandt and Snedecor (see Fisher, 1950), showed significant ($P = 0.01$) heterogeneity between progenies from green and yellow affected groups, while progenies within each group were homogeneous (P green = $0.7-0.5$, P affected = $0.3-0.2$). Progenies from affected and green Climax plants showed more extreme differences (see table 2a).

A more sensitive test of variation between affected plants was given by crosses to unaffected varieties. Thus significant ($P = 0.01$) heterogeneity was shown between the three families of Climax $\times$ Perle de Prague given in table 6, and similar variation was observed in other crosses.

(v) *Intra-individual variations*

The family totals of table 6 each represent the sum of two progenies, designated sub-families, raised from seed gathered from separate inflorescences of one plant. The pairs of sub-families of crosses 16 × 18 and 34 × 25 were heterogeneous ($P = 0.05-0.02$ and 0.01) while sub-families from 120 × 56 were homogeneous ($P = 0.5-0.3$). Therefore not only individual plants of an affected variety but also the separate

TABLE 7
*Percentages of affected seedlings in sub-families of three crosses
and their reciprocals*

Cross			Reciprocal		
	No. seedlings	Percentage affected <i>a</i>	No. seedlings	Percentage affected <i>b</i>	Difference <i>a-b</i> *
Climax × Perle de Prague .	65	100	25	100	0
	78	98.9	31	96.8	-1.1 2.1 3.2
Family totals .	143	99.3	56	98.2	-1.1
Climax × Talisman .	32	78.1	29	93.1	-15.0
	21	81.0			-12.1
	60	98.3			5.2
Family totals .	113	89.4	29	93.1	-3.7
Climax × Royal Sovereign	42	4.8	42	33.3	-28.5
	52	23.1			-10.2
	30	26.7			-6.6
Family totals .	124	17.7	42	33.3	-15.6

* Differences are obtained within families by subtracting each value in column *b*, in turn, from each value in column *a*.

parts of one plant gave wide variations in the transmission of June Yellows. The variation is related to symptom severity, for green plants of Climax and Mme. Moutôt gave fewer affected progeny than did affected plants, and a plant with extreme symptoms gave more affected progeny than plants with less extreme symptoms (see table 6). Sub-families with high proportions of affected seedlings tended to be more homogeneous than sub-families showing a lower disease incidence.

(vi) *Inter-sexual variations*

Previous evidence relating to differential sexual transmission of June Yellows is contradictory, for while McWhirter (1955) obtained

greater transmission by female gametes of Climax, Williams (1955) found increased transmission by male gametes of Blakemore. Valid comparisons to show sexual differences can be made only between reciprocal crosses made in one year and involving the same individual plants. Families planted in 1957 are arranged in table 7 to give the comparison. Heterogeneity was found only within Climax $\times$ Talisman ($P = 0.01$), Climax $\times$ Royal Sovereign ($P = 0.05$), and between the latter cross and its reciprocal ($P = 0.05$).

Intra-individual variation, demonstrated by significant differences between sub-families within families, might also account for the differences observed between reciprocal families. Nevertheless these results, and those of other years, do suggest a tendency for greater transmission of June Yellows in the male line.

TABLE 8

Percentages of affected seedlings in out cross families six months after germination

Cross	Percentage of seedlings affected		Reciprocal
...	...	46	Tardive de Leopold $\times$ Climax
Climax $\times$ Perle de Prague .	35	6	Perle de Prague $\times$ Climax
Climax $\times$ Talisman . . .	25	0	Talisman $\times$ Climax
Climax $\times$ Huxley . . .	25	...	...
Climax $\times$ Early Cambridge	3	...	...
Climax $\times$ Royal Sovereign .	0	0	Royal Sovereign $\times$ Climax

5. DISCUSSION

The variation described above cannot be accounted for reasonably by any hypothesis of nuclear gene action, but the plasmagene hypothesis will account satisfactorily for all aspects of June Yellows inheritance.

Symptoms eventually develop in most or all plants of an affected variety. Therefore the June Yellows plasmagene either must have arisen at a very early stage in the history of the clone or must have been transmitted through gametes to the original zygote. After symptoms appear they continue to increase in severity up to the time of plant death; in Darlington and Mather's (1949) terminology the plasmagene is suppressive, *i.e.* the effect of the abnormal eventually suppresses the normal. The progressive change of phenotype suggests a gradual increase in plasmagene concentration, and it follows that at low concentrations the plasmagene is symptomless, symptoms being evoked only when the plasmagene reaches some threshold concentration.

The variation between families within a cross is satisfactorily explained by variation in plasmagene concentration between the parent plants. Similar ranges of variation would be expected to occur between crosses, unless the threshold level or rate of plasmagene

reproduction in seedlings were influenced by genes from the green alternate parent. A change only in the threshold level would result in an alteration of the time at which symptoms first appear in a family, but thereafter the rate of symptom appearance should be unaffected. By contrast a change in rate of plasmagene reproduction would affect the rate of symptom appearance and also the timing of the first appearance of symptoms. Table 8 shows the overall proportions of seedlings affected in various families about six months after germination. Crosses of Climax by Early Cambridge and Royal Sovereign gave much lower proportions than other families, while Tardive de Leopold $\times$ Climax gave a high proportion. These results suggest that an upward shift of threshold level was induced by genes inherited from Early Cambridge and Royal Sovereign, while the level may have been depressed by genes from Tardive de Leopold.

Rate of yellowing in these families was illustrated in text-fig. 1. The rate was very similar in all crosses except Climax $\times$ Royal Sovereign where it was much reduced. The shift of threshold level in Early Cambridge progenies has been achieved without a corresponding alteration in the rate of yellowing, but both level and rate were affected in Royal Sovereign progenies. Genes inherited from Early Cambridge therefore affect only symptom expression, while genes from Royal Sovereign depress plasmagene reproduction and may also affect symptom expression.

The high rate of transmission of the June Yellows plasmagene by Climax male gametes contrasts with the very low rates of plastid transmission by pollen observed in other species. Considered with the correspondence between transmission rate and symptom severity, it suggests active transfer of the particles in association with regularly transmitted bodies, *e.g.* the nuclei, rather than passive transfer in cytoplasm. However, a mechanism of this kind cannot sufficiently account for greater transmission by male than by female gametes. To explain high male transmission rates of the rogue pea character, Darlington (1949) suggested that plasmagenes might multiply to high concentrations during pollen development. However, a reduction in female transmission would have the same effect, and could be brought about by a reduction in the concentration of plasmagene associated with the egg nucleus, consequent on the repeated division of the embryo sac mother nucleus.

Williams (1955) considered June Yellows to be caused by a rogue plasmagene, but he considered that the loss of a normal plasmagene could fit the facts equally well. However, the occurrence of affected seedlings in crosses of green varieties with Climax as male parent requires the transmission of some positive, cytoplasmic factor by pollen and cannot be explained on a hypothesis of mere absence.

The mode of origin of June Yellows is of practical and theoretical interest. Darlington and Mather (1949), McWhirter (1955) and Williams supposed the particle to be a mutant form of a plasmagene

normally present in a strawberry. In this event a two-fold response to mutation is required: first a change in the action of the plasmagene, and second a release from the controls to which its multiplication is normally subject. Alternatively the plasmagene might be derived from a virus. Exogenous micro-organisms are now considered to cause some of the phenomena that were once given as examples of plasmagenes, *e.g.* *Kappa* in *Paramecium* (Beale, 1961) and CO₂ sensitivity in *Drosophila* (L'Héritier, 1958). Lister (1960) and Cadman and Lister (1960) have recently demonstrated seed and gametic passage of three soil-borne ringspot viruses in strawberry and other plants. Infectivity is no longer of prime importance in the life cycle of a virus transmitted efficiently through seed and the presence of a symptomless, non-infective mutant could be detected only by special techniques. Selection between infected plants would favour the combination of virus and host genotype that least depressed the reproductive potential of the host. It is not known whether any attenuation of infectivity accompanies gametic transmission of the ringspot viruses, but it is of interest that symptoms may be absent in infected progeny or may be of different kinds to those caused by artificial or field infection.

It is also of interest in this connection to recall the variable inheritance of the *Drosophila* CO₂ sensitivity virus. In the non-stabilised state inheritance is matrilinear and infected flies give high yields of virus particles, while in the stabilised state inheritance is ambilinear and only low virus yields are obtained on assay. Existence of the stabilised state suggests that the virus may enter a very close association with the nucleus. L'Héritier (1958) has commented on the parallel between this situation and the relationship between phages and the bacteria they infect.

Further parallels of behaviour between June Yellows and the ringspot viruses are known. Smith and Harland (1958) reported that Climax plants propagated from dormant buds of crown sections sometimes failed to show June Yellows symptoms. In a more extensive experiment I have found unaffected plants to arise from buds randomly situated on old crowns, and some of these plants have remained symptomless for three years. Lister (unpublished) has noted similar freedom from raspberry ringspot virus on propagation from infected strawberry crowns. Further, the seasonal cycle of symptom expression corresponds very closely in the two groups. It is not suggested here that the June Yellows plasmagene is directly related to a ringspot virus, but the parallels are sufficiently striking to justify new investigation of the nature of June Yellows by modern virological techniques. Until then it may be unwise to attempt a choice between the alternative hypotheses but at the present state of knowledge that of virus origin is more attractive.

The occurrence of affected seedlings in progenies of Talisman shows that June Yellows can be transmitted and manifested in seedlings

while the plasmagene is still below threshold concentration in the parent variety. The longest known latent period was recorded by Darrow (1934), who stated that the variety Haverland first showed symptoms more than fifty years after it was raised from seed. It is possible that the threshold concentration might never be reached in some varieties. Progeny testing by selfing has long been advocated (Demaree and Darrow, 1937) as a method of discovering these latent carriers; but where carrier varieties possess June Yellows inhibiting genes, crossing to a variety like Tardive de Leopold having genetic factors favourable to June Yellows development, would provide a more certain test.

June Yellows has occurred in varieties, like Climax, with no record of the disease in their pedigree. A strawberry breeding programme therefore must be pursued with an awareness that June Yellows may occur without warning in many related selections. Protection for the breeder lies in the use of diverse parental material and selection within more than one line. This procedure cannot always be reconciled with the breeding objective and affected varieties necessarily may be bred from where, for example, genes for disease resistance are found only within affected or suspect lines. In this case the breeder would be advised to restrict the use of such material to the maternal line, when transfer of the plasmagene may be slightly restricted.

Accelerated fading of symptoms in plants exposed to warmer temperatures suggests the possibility, so far uninvestigated, of reducing disease incidence in seedlings by altering the environment at the time of gametogenesis, fertilisation or early zygote development. However, the outlook is not promising for heat treatment, for I have found that no permanent symptom modification occurred in Climax plants after growth at *ca.* 80° F. for six months, and Chambers (unpublished) found no permanent change in symptoms after short exposure at *ca.* 100° F. in a heat therapy cabinet.

Protection for the grower, when a widely grown variety becomes affected, would be afforded by the ready availability of equally suitable alternative varieties. Affected plantings could then be grubbed out after the normal commercial period and replanted with an unaffected variety. Sufficient suitable varieties are not yet available in Britain and the sudden decline of a popular variety would still cause economic loss, although fortunately not on the same scale as when Climax became affected.

6. SUMMARY

Two June Yellows affected varieties and six green varieties were selfed and reciprocally intercrossed, and their progenies were observed over a period extending to four years. Both affected varieties gave affected progenies, and one green variety (Talisman) also gave some affected seedlings. Green seedlings in these families were likely to begin showing symptoms throughout the period of observation.

Significant differences could be found between the proportions of affected seedlings in families from different plants of a clonal variety and even from different inflorescences of single plants. Increased proportions of affected seedlings were observed from similar crosses repeated in successive years.

June Yellows is not transmitted by grafting and no insect vectors are known.

Inheritance cannot be interpreted readily on a mendelian basis, but the hypothesis of plasmagene control convincingly accounts for all aspects of heredity. The concentration of a plasmagene would be expected to vary from cell to cell and this fluctuation would give rise to the observed variation in symptom expression and variations in hereditary transmission of the disease.

Carrier plants do not show symptoms until the plasmagene has multiplied to a threshold concentration. Multiplication rate and threshold concentration are both influenced by the genotype of a carrier plant.

The June Yellows plasmagene may be a mutant cell constituent of strawberry but more probably it is an exogenous, virus-like particle. High transmission of the disease through pollen indicates that the plasmagene may exist in intimate association with cell nuclei.

Some implications of this theory are discussed in relation to strawberry breeding and growing.

7. REFERENCES

- BEALE, G. H. 1961. The role of cytoplasm in inheritance. *Sci. Prog.*, 49, 17-35.
- CADMAN, C. H., AND R. M. LISTER. 1960. Some aspects of recent work on soil-borne viruses. *Proc. 4th Conf. Potato Virus Dis. Braunschweig*, 1960, pp. 17-21.
- CLARK, J. H. 1930. Non-infectious chlorosis of the strawberry. *New Jersey Agric. Exp. Sta. Ann. Rept.* 1930, 215.
- DARLINGTON, C. D. 1949. Genetic particles. *Endeavour*, 8, 51-61.
- DARLINGTON, C. D., AND K. MATHER. 1949. *The Elements of Genetics*. George Allen & Unwin Ltd., London.
- DARROW, G. M. 1934. Notes on variegated leaf troubles in strawberries. *Plant Dis. Repr.*, 18, 26-29.
- DEMAREE, J. B., AND G. M. DARROW. 1937. Leaf variegation in strawberry not considered a virus disease. *Plant Dis. Repr.*, 31, 400-403.
- FISHER, R. A. 1950. *Statistical Methods for Research Workers*. 11th ed. Oliver and Boyd, Edinburgh.
- L'HÉRTIER, PH. 1958. The hereditary virus of *Drosophila*. *Adv. Virus Res.*, 5, 195-245.
- LISTER, R. M. 1960. Transmission of soil-borne viruses through seed. *Virology*, 10, 547-549.
- MCWHIRTER, K. G. 1955. "June Yellows", a cytoplasmic mutation in the cultivated strawberry. *Heredity*, 9, 151-152.
- REID, R. D. 1951. June Yellows in Climax plants. *Fruit Grower*, pp. 1056-1057.
- REID, R. D. 1956. Strawberry breeding at Auchincruive. *Sci. Hort.*, 12, 140-146.
- SMITH, M. C., AND S. C. HARLAND. 1958. Can this mean Climax free of Yellows? *Grower*, 49, 549-550.
- WILLIAMS, H. 1955. June Yellows: a genetic disease of the strawberry. *Jour. Genet.*, 53, 232-243.

Plate Ia

Plant of Auchincruive Climax in early spring with chlorosis and variegation
typical of June Yellows.

Plate 1b

Two sub-clones of Auchincruive Climax in a replicated planting.
June Yellows has caused extreme stunting in one sub-clone.