A STUDY OF THE CYTOGENETICAL EFFECTS OF 2,4-D ON BARLEY

E. N. LARTER

T H E U N I V E R S I T Y O F A L B E R T A

A STUDY OF THE CYTOGENETICAL EFFECTS OF 2,4-D ON BARLEY

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Edward Nathan Larter

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ABSTRACT

Mitotic and meiotic responses to 2,4-D were studied in barley.

Separate ten-foot rows were treated on the same day, one with eight the other with 16 ounces of acid equivalent per acre. Treatments were repeated at three-day intervals.

Cytological examination of meristems of treated seedlings disclosed chromosomal multiplication, stickiness and fragmentation. Abnormalities tended to be localized in separate tillers and reached maximum expression about nine days after treatment.

A meiotic study of treated plants revealed that the most frequent abnormality that occurred in all dates of treatment was cells with sticky chromosomes. Chromosomal fragmentation and aneuploidy, however, was also commonly found. Maximum frequency of cytological abnormality occurred in material treated shortly before microsporogenesis.

In an analysis of pollen from treated plants, the presence of pollen grains that were smaller and/or poorly filled with starch might be evidence that such were results of affected microsporocytes.

Abnormality frequencies in pollen mother cells and pollen grains were highly correlated.

Rings and chains of four chromosomes were observed in two treated plants, while in another, a centric fragment was found to occur.

The cytological results from the T1 generation were similar to those found in the treated material. It is postulated that the effects of 2,4-D were transmitted mainly as a chemical disturbance or stimulus, except in the case of the quadrivalents and the centric fragment.

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INTRODUCTION

From the time that 2,4-D was first introduced as a chemical for weed control, a large number of field studies have been conducted on methods of application, and on its physiological and morphological effects. As a result of such studies, the action of this herbicide as a growth regulator or stimulator has been demonstrated. Moreover, it has been found that excessive stimulation with subsequent death is much more marked in dicotyledonous than in monocotyledonous plants. As a result, it is used more and more extensively for the control of broad-leaved weeds in cereal crops.

From time to time, attention has been drawn to gross morphological effects in treated plants by both farmers and scientists. These abnormalities have consisted for the most part of twisted leaves, stems and spikes, extreme spike malformations, and sterility. Usually the explanation has been that spraying was done at susceptible stages of the plants or at excessive rates.

The literature shows that studies on the mitotic effects of 2,4-D are rather limited and confined to work under laboratory conditions. While these studies point to probably cytogenetic effects of this chemical, a study under field conditions would be necessary to determine if such effects are transmissible. Chromosomal or genetic disturbances would have far reaching effects in altering the genetic constitution, especially of presumably pure seed stocks.

It is the purpose of this paper to report and discuss the investigations conducted to determine:

1. Whether cytological abnormalities may be produced by field applications of 2,4-D.

2. Whether the disturbances that may be produced are transmissible.

LITERATURE REVIEW

Mode of Action of 2,4-D.

Many reports of the possible mode of action of 2,4-D are available (2, 4, 16, 17, 26, 27). Many aspects in relation to its toxic action, however, are still not fully understood.

It is generally agreed by workers in this field that most dicotyledonous plants absorb 2,4-D through their leaves more readily than do monocotyledonous plants. Blackman (2), and Mitchell (16), reported that penetration appears to be directly through the leaf cuticle rather than through the stomata.

Once within the plant, transport of the chemical appears to be confined to living phloem tissue (2, 17). Mitchell and Brown (17), and Weaver and DeRose (27), however, found evidence to show that upward transport in the xylem is also possible. Once the chemical is at the site of toxic action, biochemical differences that are known to exist between plants may be responsible for its selectivity (2).

According to Mitchell and Brown (17), and Weaver and DeRose (27), any factor that generally affects the movement of photosynthetic material, also governs the rate of stimulus movement within the plant. Among such factors may be included root competition, moisture supply, and light (4, 26).

Blackman (2), and Mitchell (16), found 2,4-D to cause an upset of the carbohydrate balance within a susceptible plant. Reserve carbohydrates gradually decrease, while reducing sugars at first accumulate and then diminish.

Morphological and Physiological Effects of 2,4-D.

The data being gathered concerning the physiological and morphological effects of 2,4-D with respect to practical field observations is voluminous and ever increasing. Friesen (11), and Olson, et al (20), reported that the treatment of wheat and barley at various stages of growth caused a significant reduction in yield at two widely separated periods. These stages of growth were an

interval extending from emergence to a height of about five inches, and again during the shot blade stage.

Erickson, et al (10), and Mitchell (16), reported increased protein content of the grain when wheat seedlings were sprayed with field applications of 2,4-D. Erickson, et al (10), found a direct relationship between concentration used and percentage protein of the grain. Although no yields were recorded by him, he believed that a relationship existed between observed stunting of the plants and protein content of the grain, especially at higher concentrations.

In similar work carried out with barley, Corns (3) reported finding that rates of three to six ounces of acid equivalent of 2,4-D per acre did not affect protein content. There did occur a slight protein percentage increase (0.2% - 0.6%), when applications of over nine ounces were used. At the higher levels, however, a reduction of over five bushels per acre resulted.

Cotton seedlings grown from seed bolls present on the plant at the time of 2,4-D treatment, were found by McIlrath (15), to exhibit varying degrees of leaf malformation. Lowered germination percentages also accompanied the morphological abnormalities at higher rates of application.

He also reported that the transmission of such anomalies via the seed embryos continued for a period of eight weeks post treatment.

Cytological Effects of 2,4-D and Related Herbicides.

Although there has been a limited amount of investigation on the effects of various herbicides upon mitotic behaviour (7, 8, 19, 21), no such studies have been reported conducted on meiotic behaviour. Moreover no attempt has been made to determine whether cytological disturbances that may be induced by these chemicals could be transmissible.

Nygren (19), while studying root tip mitoses affected by several chemicals, observed similar abnormalities produced by the three herbicides, 2,4-D, 2-methyl, 4-chlorophenoxyacetic acid (methoxone), and 2,4,5-trichlorophenoxyacetic acid. C-mitotic pairing, stickiness, and chromosomal fragmentation were the main disturbances observed. Difficulty in obtaining satisfactory staining of affected chromosomes lead Nygren to believe that toxic action of the chemicals caused a disturbed nucleic acid cycle.

Doxey and Rhodes (8), and Ryland (21), from their observations of root tips treated with two of the three chemicals used by Nygren (19), reported similar findings. Ryland (21), however, did not observe stickiness and fragmentation during her investigations. It is significant nevertheless, that both workers parallel the action of the herbicides upon chromosomal behaviour with that caused by X-rays, and to conditions found in tumerous tissue cells as reported by Koller (12).

Isopropyl phenyl carbamate was found by Doxey (7), to have an effect upon root mitoses similar to that of colchicine (14), and certain herbicides (8, 19). Chromosomal contraction resulting from the action of this chemical was not as apparent, however, as that resulting from effects of some herbicides (8).

Cytological Effects of Some Insecticides and Antibiotics.

From results reported to date, the capability of these chemicals to induce changes in genetic make-up is evident (9, 25, 28).

The effects of D.D.T. insecticide upon plant mitoses, as reported by Vaarama (25), clearly resemble those of organic herbicides (7, 8, 19, 21). Although acqueous solutions of this chemical produced only weak c-mitotic effects, alcoholic solutions gave rise to stickiness and fragmentation of chromosomes. Formation of nucleoli became progressively more prevalent from the metaphase to telophase stages of mitosis.

The gamma isomer of benzine hexachloride apparently exerts its main influence upon the cell spindle rather than the chromosomes directly. Doxey and Rhodes (9), found that the spindle development at metaphase of mitosis was suppressed by the action of this chemical. The metaphase chromosomes were as a result, scattered throughout the cell. Chromosomal fragmentation, however, did not appear to be caused by this chemical.

Certain antibiotics have also been found capable of inducing mitotic abnormalities. Investigations by Wilson and Bowen (28), revealed that concentrations of

50 to 100 p.p.m. of Streptomycin, Aureomycin, and four other related antibiotics were capable of causing c-mitotic abnormalities. Concentrations of 150 p.p.m. induced severe stickiness and clumping of mitotic chromosomes. The affected cells failed to recover after termination of the various treatments.

Cytological Effects of Some other Chemicals.

Many chemicals other than those with herbicidal and insecticidal properties, have been studied for their ability to cause chromosomal changes.

The action of colchicine is well known as a polyploidizing agent through its specific action of spindle suppression (14). Kostoff (13), reported that the action of acenaphthene upon mitotic behavior is similar to that of colchicine.

Other chemicals are more drastic in their action upon the cell. Darlington and Koller (5), and Novick and Sparrow (18), reported that the nitrogen and sulfur mustards produce serious chromatid breakage and stickiness.

These workers believe that such chemicals cause an overcharge of nucleic acid on the chromosomes.

Auerbach, et al (1), reported lethal mutations to occur in Drosophila from the effects of mustard chemicals, as detected by the C 1 B method. Although Auerbach (1) carried out similar experiments with other chemicals including carcinogens, no mutations were found to occur.

Demeric (6), using methods similar to those used by Auerbach (1), later found dibenzanthrene to be a carcinogen with lethal mutagenic properties.

MATERIALS AND METHODS

Olli barley was used as the experimental material. Because of the small chromosome number (n = 7), cytological observations are facilitated especially if a large number of cells have to be analyzed in order to establish treatment effects.

A 2,4-D Ethyl ester (Weedone) containing 34.6% dichlorophenoxyacetic acid by weight, was applied by a pressure tank sprayer. Two concentrations were used, viz., eight ounces and 16 ounces of acid equivalent per acre. Both concentrations were applied in the form of a water spray at the equivalent rate of 80 gallons per acre. Single 10-foot space-planted rows were treated once with either the high or low concentration. Treatments were applied at three-day intervals so that the first plot was treated at emergence, and the fifteenth, or final plot at a period shortly after heading.

To protect neighboring plots from spray drift, canvas shields were erected surrounding each row receiving treatment. Stages of development of untreated plots, as well as observable effects of the chemical on previously treated plots were recorded at each date of spraying.

An attempt was made to study the effects of the chemical on somatic tissue by the cytological examination of mitoses of stem meristems. On the twenty-first day following emergence, 20 seedlings were collected from material treated with each concentration at the seven previous treatment dates. All tillers from each plant were cytologically examined separately.

At microsporogenesis, ten plants from each date and concentration of treatment were used for study. Since microsporogenesis in the main tillers was observed to occur at about the time of the twelfth treatment, only material from that date and the previous treatment dates were used for meiotic studies. Two tillers from each plant were examined and the number of microsporocytes per tiller ranged from 150 to 350.

Several spikes were also selected from each concentration and treatment date for pollen grain analysis. Two counts were made from each spike and an attempt was made to analyse only those florets that were at approximately the same stage of development. Six thousand to 8,000 pollen grains were examined from each treatment.

Seed from all spikes of 13 treated plants exhibiting varying degrees of cytological abnormality

was seeded in head rows in the greenhouse in October, 1951. An attempt was made to examine cytologically one tiller from each of the five plants per head row.

Unfortunately, greenhouse conditions were poor during the winter months, and as a result morphological studies of the T1 generation were thought inadvisable.

The cytological analysis of all treated and T1 material was compared with that of control material.

Material to be examined at microsporogenesis, as well as that used for pollen grain analysis, was fixed and stored in 6:3:1 Carnoy's fluid A. The acetocarmine smear method as outlined by Smith (24), was used for pollen mother cell preparations. An acqueous solution of iodine in potassium iodide was used for pollen grain preparations. It was found that, unlike alcoholic solutions of iodine, the acqueous solution allowed sufficient time for slide examination before excess evaporation occurred.

Stem meristem material was fixed in Farmer's fluid and the Feulgen smear method was used for slide preparations.

Chromosome examination was made at a magnification of 540 diameters and photomicrographs were taken at a magnification of 540 or 1,080 diameters.

EXPERIMENTAL RESULTS AND DISCUSSION

Cytological Data from Treated Material

Mitotic Behaviour in Stem Meristems.

It was found that the total number of actively dividing meristematic cells from plants within any one concentration were too small to establish a valid percentage abnormality. As a result, the combined analysis of both concentrations were used to express the percentage abnormality as found for each of the treatment dates. The results for both treated and control material are presented in Table 1.

The action of 2,4-D appeared to be localized to the extent that only those tillers that came in actual contact with the chemical were affected. On the same plant, therefore, there were some tillers with a high frequency of abnormal cells and others in which the divisions were normal. An extreme example was found in a

Table 1

Frequency of Mitotic Aberrations in Treated Plants Sampled for Cytological Purposes 21 Days After Emergence.

Date of treatment	No. plants analysed	No. normal cells	No. aberrant cells			% abnormality
			Sticky	Polyploid.	Fragment.	
Check	3	309	0	3	0	1.0
Emergence	3	135	13	3	0	10.6
3 days after	2	150	7	2	4	8.0
6 days after	2	87	10	0	0	10.3
9 days after	2	217	11	2	13	10.6
12 days after	2	107	13	29	1	28.7
15 days after	3	550	30	6	2	6.5
18 days after	2	502	11	0	1	2.3
Total	19	2057	95	45	21	

plant treated at emergence. Of 197 mitotic cells examined from two tillers of this plant, 33.3% were abnormal in the first tiller, and only 1.1% were abnormal in the second. There appeared to be, therefore, no transfer of chemical or stimulus from tiller to tiller. Mitchell and Brown (17), in their investigations of movement of 2,4-D within the bean plant, report similar findings.

The results in Table 1 bring out one very striking effect of the chemical. The treatments applied from emergence to nine days thereafter, caused a similar frequency of abnormal mitoses. In material treated 12 days after emergence there occurred a marked increase in frequency of abnormality, and then as a result of later treatments, a rapid decrease. This can be explained as follows:

Since material from all treatments was sampled 21 days after emergence, the effects of the chemical by that time probably had become constant in material treated on the first four dates. In material treated 12 days after emergence, (nine days before sampling), the effect and build up of abnormal mitoses was at a maximum. This observation coincided very closely with morphological effects (wilting and leaf curling), which were most extreme approximately nine days after treatment. Since the chemical appears to cause progressively more abnormalities up to nine days after

application, in material treated shortly before sampling one would expect fewer abnormal mitoses. This is confirmed by the results from treatments applied 15 and 18 days after emergence, (six and three days respectively before sampling).

Such periods of increasing and decreasing frequencies of abnormal divisions suggested that many affected cells did not take part in further divisions. Nevertheless, as will be shown later, meiotic irregularities reappeared in early treated plants. It must be interpreted that either cells with a changed chromosomal complement continued to function, or that the chemical remained active within the plant.

The types of aberrations that occurred were similar to those observed in treated root tips by Doxey and Rhodes (8), and Nygren (19).

With exception of one treatment date, sticky chromosomes were the most frequent and consistent abnormality. Polyploidy, however, occurred very frequently at one date of treatment. Although tetraploidy was the most commonly observed degree of duplication, a few octaploid cells were found, Fig. 2. In addition, there were observed cells with varying degrees of fragmentation and pycnosis.

Control material was extremely regular with only three abnormal cells from a total of 312 examined.

Meiotic Behaviour in Pollen Mother Cells

The results from the examination of pollen mother cells from treated plants are presented in Table 2. The percentage abnormality for the two concentrations are combined and the mean percentage expressed for each treatment date.

Two plants containing quadrivalents, and one containing a centric fragment will be discussed separately. The cytological results from these plants, therefore, are not included in Table 2.

It is perhaps not surprising that the 16-ounce concentration caused a significantly higher mean percentage abnormality than did the eight-ounce concentrations. ($t = 5.38$, $d.f. = 288$). Since the cytological results were expressed as a percentage abnormality, it was thought advisable to transform the data prior to calculating tests for significance. The angle method was used, therefore, to make all transformations.

Attention should first be drawn to the variability of frequency of cytological aberrations between plants of the same treatment date. It was later found that the variability extended to different tillers of the same plant and even to different florets of the same spike. Koller (12), reports

Table 2

Frequency of Meiotic Aberrations in Treated Plants

Time of treatment	No. plants examined*	Norm. cells	Aberrant cells						Mean % aberration	% range of abnorm.
			Sticky	Fragments	Aneuploid	Mult. spor.	Polyp.	Univ.		
Check	16	4634	27	18	5	0	2	1	1.1	0.4-2.4
Emergence	20	6771	130	70	17	8	0	2	3.2	0.5-9.3
3 days after	17	5931	93	47	12	11	7	2	2.8	0.0-13.9
6 " "	13	4449	171	86	20	12	0	0	6.1	0.6-23.4
9 " "	16	5669	142	81	15	10	2	0	4.2	0.7-13.8
12 " "	15	7812	244	87	29	17	2	3	4.7	0.0-10.3
15 " "	20	7391	162	57	25	16	12	4	3.6	1.0-11.0
18 " "	15	4210	121	88	20	12	3	1	5.5	0.4-24.1
21 " "	21	9944	214	97	18	16	4	1	3.4	0.0-18.8
24 " "	20	9513	373	174	53	14	2	2	6.1	0.3-43.5
27 " "	20	5608	361	154	32	17	10	1	9.3	1.2-50.9
30 " " **	18	5662	155	89	38	8	7	1	5.0	0.0-22.2
33 " " **	15	11239	353	96	35	14	4	3	4.3	1.0-50.5

* Two tillers per plant.

** Approximate period of sporogenesis.

similar findings in naturally occurring tumerous tissues. He found aberrant mitotic cells to vary quantitatively from one tumour to another as well as within different regions of the same tumour.

While there was no period at which the mean percentage abnormality equalled that of controls, the highest frequency occurred just prior to, or during microsporogenesis. This stage at which spraying resulted in maximum meiotic disturbance coincides closely with that at which Olson et al (20), obtained a significant yield reduction in 2,4-D treated barley.

The degree of morphological disturbance resulting from each treatment was not studied in the present investigation. The treatment date that caused maximum meiotic disturbance, however, did not appear to cause excessive numbers of morphologically abnormal plants. Abnormal spikes, (Fig. 1), and onion-like leaves occurred consistently throughout all treatments. These results, therefore, differ from those of McIlrath (15), who found greatest morphological disturbance to occur in cotton plants sprayed at anthesis.

Fig. 1

Abnormal spikes of 2,4-D treated barley.

Stickiness of chromosomes was the most frequently observed irregularity. The extent of stickiness ranged from a single sticky pair, (Fig. 3), to a clumping and agglutination of the entire chromosomal complement within any one cell, Fig. 5. In cases in which stickiness was severe, it was noted that the cytoplasm was vacuolated and disintegrated, Figs. 4 and 5.

Microsporocytes containing acentric fragments were observed frequently. Many such fragments were not entirely separated from the mother chromosome, but remained attached by a fine chromatin thread, Fig. 6. No doubt, further chromosomal movement would cause breakage of the connecting material allowing the fragments to be scattered throughout the cytoplasm, Fig. 7. The frequency of cells with fragments so closely paralleled that of cells with sticky chromosomes, that a highly significant correlation was found to exist between the two types of aberrations, ($r = 0.83$; $t = 4.71$ at d.f. = 10). It may be that the primary action of the chemical was to cause stickiness by upsetting the nucleic acid charge within the chromosomes. Fragmentation occurred when cell division forces were exerted upon such sticky chromosomes.

Multiploid sporocytes, as described by Smith (23), occurred consistently in all treated material at microsporogenesis, Fig. 8. The chemical apparently had the effect of causing several pollen mother cells to fuse, resulting in multiples of the normal chromosomal complement to congregate at one common metaphase plate. The plants in which Smith (23) found this irregularity to occur, frequently produced lowered seed set.

Aneuploidy also occurred frequently. A cell was classified as being aneuploid only after it was established that the cell wall was entirely intact. Most aneuploid cells were observed to have six or eight bivalents, Fig. 9. Analysis of anaphase I and later of anaphase II, revealed that such cells were capable of further divisions, Fig. 10. It is, of course, questionable whether aneuploidy in a diploid species would be transmitted. If aneuploidy is transmitted, probably only aneuploid female gametes would be functional.

A low frequency of cells with lagging chromosomes was also found at anaphase I and anaphase II, Figs. 11 and 12.

Although some polyploid cells were observed at meiosis, at no time was the duplication observed greater than a tetraploid, Fig. 13. Since such cells were not found consistently, the chemical or its stimulus appeared to remain active within the plant.

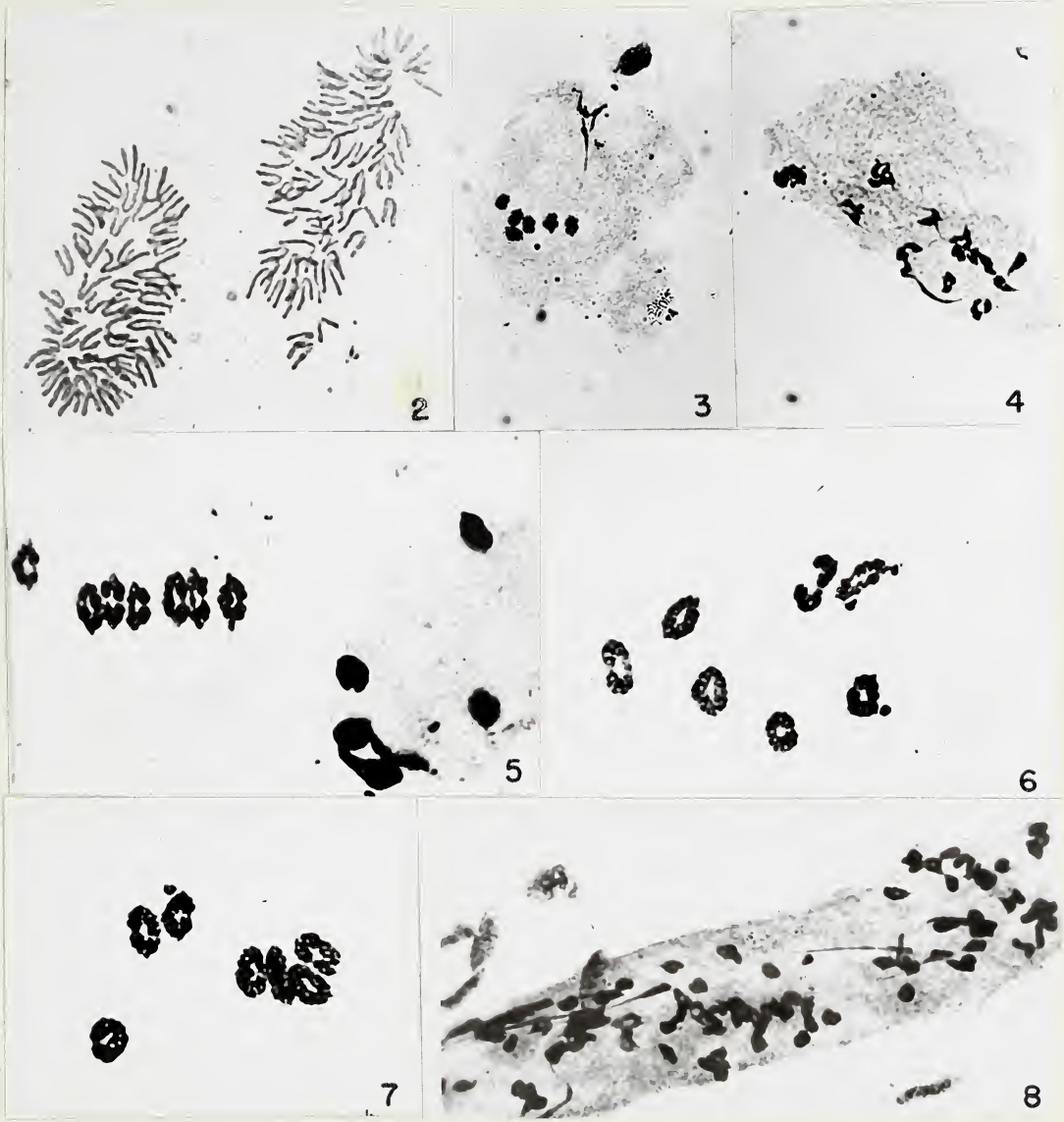

Plate I

Legend to Plate I

Cytological abnormalities in treated and T1 plants.

Fig. 2. Mitotic anaphase. Octaploid cell.

Fig. 3 and 4. Metaphase I. Cells with sticky chromosomes.

Fig. 5. Cell with 7 normal pairs, a second cell with chromosomes completely agglutinated.

Figs. 6 and 7. Metaphase I. Cells with attached and acentric fragments.

Fig. 8. Metaphase I. Multiploid sporocyte.

Magnification:

Figs. 3, 4, and 8, at 540 diameters;

all others at 1080 diameters.

The occurrence of a few polycentric cells at meiosis, suggests that the influence of the chemical was in part upon the spindle, Fig. 14.

Cells with univalents were observed at such a low frequency in all treatments, that their occurrence could not be regarded as a result of the action of the chemical. All cells observed to be affected in this way, contained 6"2', Fig. 15.

Two plants from treatments applied 21 and 27 days after emergence respectively, were found to contain rings and chains of four chromosomes, Figs. 16 and 17. In the first of these plants, both tillers revealed quadrivalents in 63.4% and 64.4% of the pollen mother cells examined respectively. In the second plant, of which it was possible to examine only one tiller, 58.7% of the cells were affected. A single cell from this tiller was observed containing a ring of 6, Fig. 18.

Although an accurate count was not made of the proportions of cells with open and zig-zag quadrivalents, approximately only 10% were of the latter type, Fig. 19. Undoubtedly the tillers in which rings and chains were found had reciprocally translocated chromosomes. The proportions of the two types of quadrivalents found, however, are not similar to those found by Smith (22). He reported finding a preponderance of zig-zag rings in microsporocytes of four barley plants affected with a reciprocal translocation.

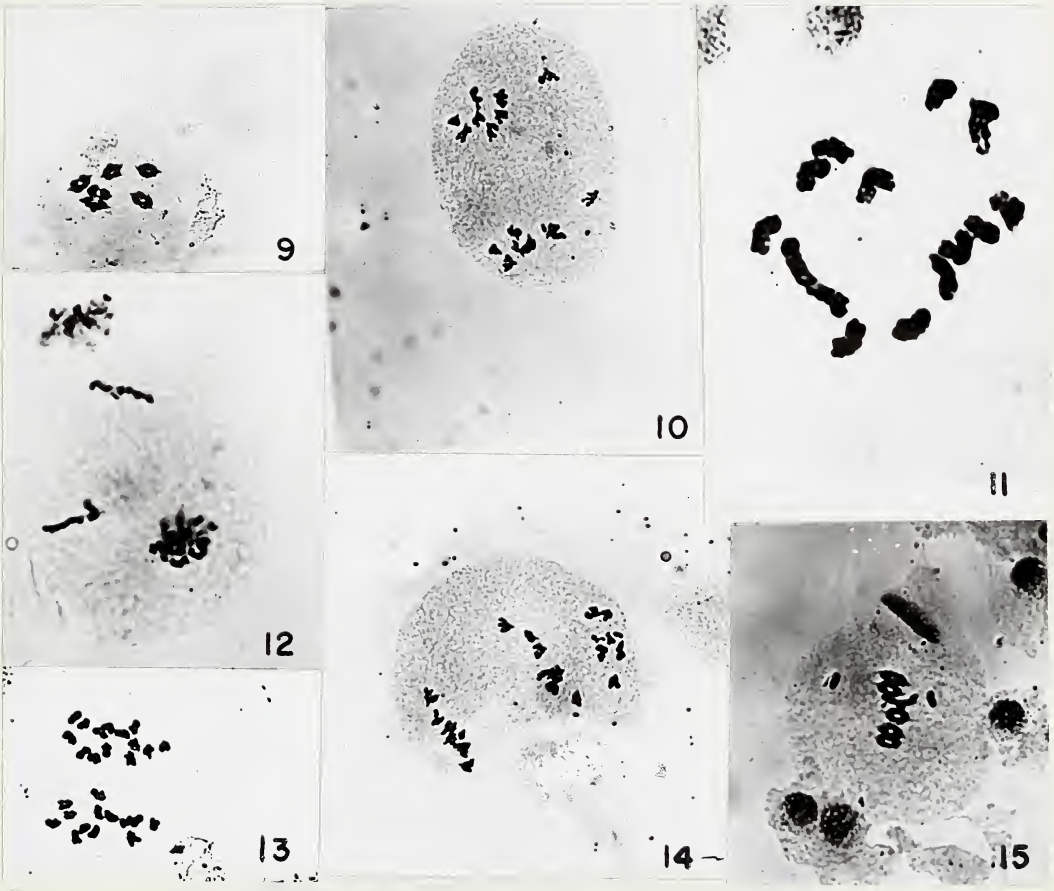

Plate II

Legend to Plate II

Cytological abnormalities in treated and T1 plants

Figs. 9 and 10. Aneuploid Metaphase I and Anaphase I respectively.

Fig. 11. Anaphase I. Tardy disjunction.

Fig. 12. Anaphase II. Lagging chromosomes.

Fig. 13. Anaphase I. Tetraploid cell.

Fig. 14. Anaphase I. Polycentric cell.

Fig. 15. Metaphase I. Cell with 6" 2'.

Magnification:

Figs. 11, and 12, at 1080 diameters;

all others at 540 diameters.

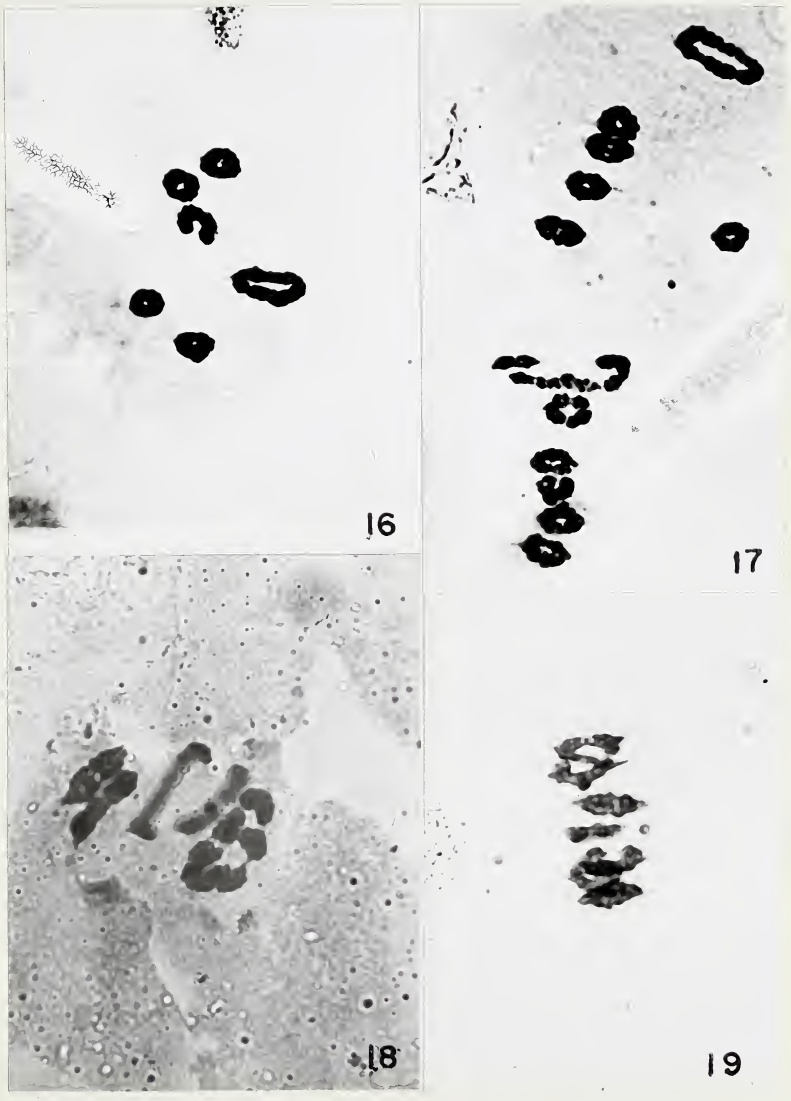

Plate III

Legend to Plate III

Cytological abnormalities in treated and T1 plants

Figs. 16 and 17. Metaphase I. Cells with 5"R"" (open).

Fig. 18. Metaphase I. Cell with 4"R"""".

Fig. 19. Metaphase I. Cell with 5"R"" (Zig-zag).

Magnification: - 1080 diameters.

It was expected as a result of the high frequency with which cells with open type rings occurred, that a high degree of sterility would occur in remaining spikes of affected plants. When the first of these plants was harvested, however, it was found that only four of the nine spikes present on this plant exhibited partial sterility. The percentage of sterile florets in each affected spike was 41.4, 46.3, 48.0, and 50.9%, respectively.

The second plant was found to exhibit no degree of sterility in spikes from remaining tillers.

There is little doubt that the chromosomal translocations were induced by the chemical, since they were found both cytologically and by observable morphological effects only in certain tillers.

A large proportion of the cells containing 7^{II} were observed to have two open bivalents. If a small segment was involved in the interchange, cells with such configurations would be expected along with those containing rings and chains of four chromosomes. Moreover, such open bivalents with translocated segments could be orientated in such a way that balanced chromosomal complements could be formed at disjuction. Functional gametes would be produced in this way. As a result, relatively good fertility would be obtained despite the high proportion of microsporocytes containing open rings of four chromosomes.

A single plant containing a centric fragment was also found in the treated material. The two tillers that were cytologically examined from this plant, revealed the fragment to be present in 87.4% and 88.7% of the pollen mother cells examined at metaphase I. Its behaviour at meiosis was similar to that of a univalent chromosome and it was possible to follow its movements through each stage of division, Figs. 20 and 21.

During the formation of quartets, the fragment was often excluded from the main nucleus and was then observed as a micronucleus in either one or two of the four sporocytes, Fig. 22.

Pollen Grain Analysis

The cytological analysis of pollen grains from treated plants, further substantiated the earlier finding that a number of affected microsporocytes remained functional. It is possible that undersized and starch deficient pollen grains, (Fig. 23), resulted from such affected microsporocytes. It is true that the starch-iodine test cannot be regarded as a positive indication of pollen fertility. Nevertheless, it was found that the correlations between frequencies of abnormal microsporocytes and affected pollen grains were highly significant for both concentrations of the chemical, Figs. 25 and 26. ($r = 0.79$ for the eight-ounce, and 0.84 for the 16-ounce concentrations).

The mean percentage of abnormal pollen from control plants was found to be only 1.3%.

A study of pollen mitosis of treated plants undoubtedly would have supplied definite information as to the exact nature of persisting aberrations.

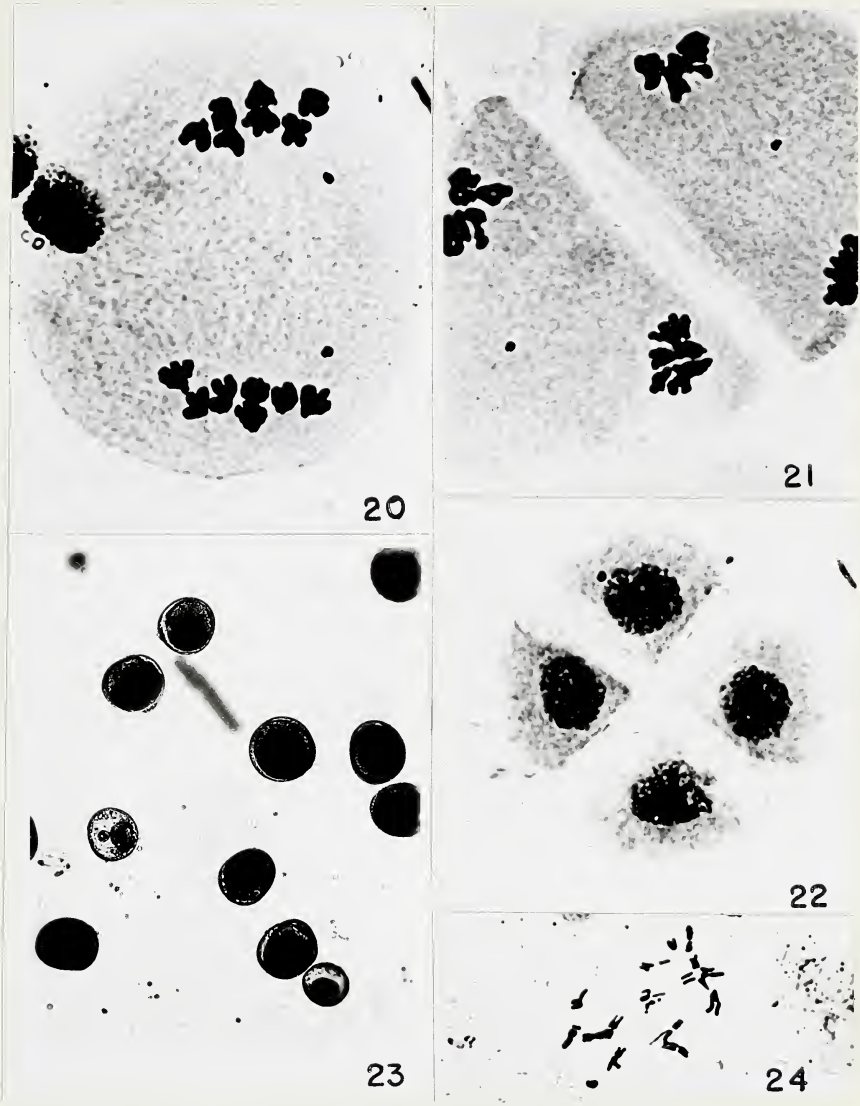

Plate IV

Legend to Plate IV

Cytological abnormalities in treated and T1 plants

Fig. 20. Anaphase I. Division of centric fragment.

Fig. 21. Anaphase II. Division of centric fragment.

Fig. 22. Quartet stage. Micronucleus in one sporocyte.

Fig. 23. Normal and abnormal pollen grains.

Fig. 24. Mitotic metaphase. Abnormal chromosome pairing.

Magnification:

Fig. 23 at 240 diameters.

Figs. 22 and 24 at 540 diameters.

Others at 1080 diameters.

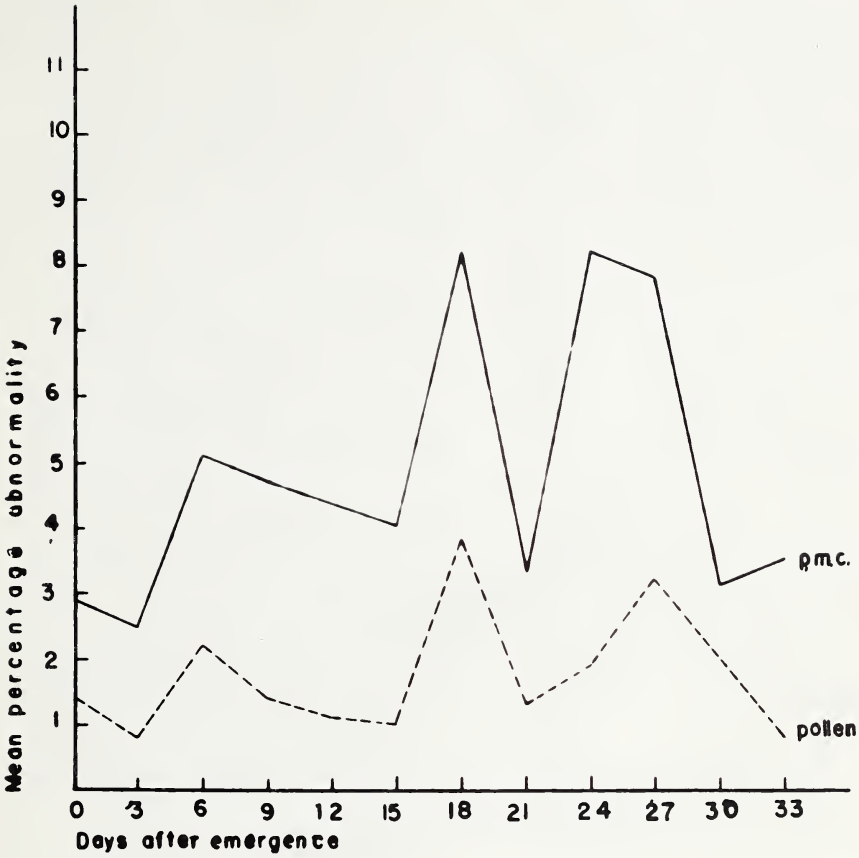

Fig. 25

Abnormality of pollen mother cells and pollen resulting from 8-ounce 2,4-D treatments of barley at different stages of development.

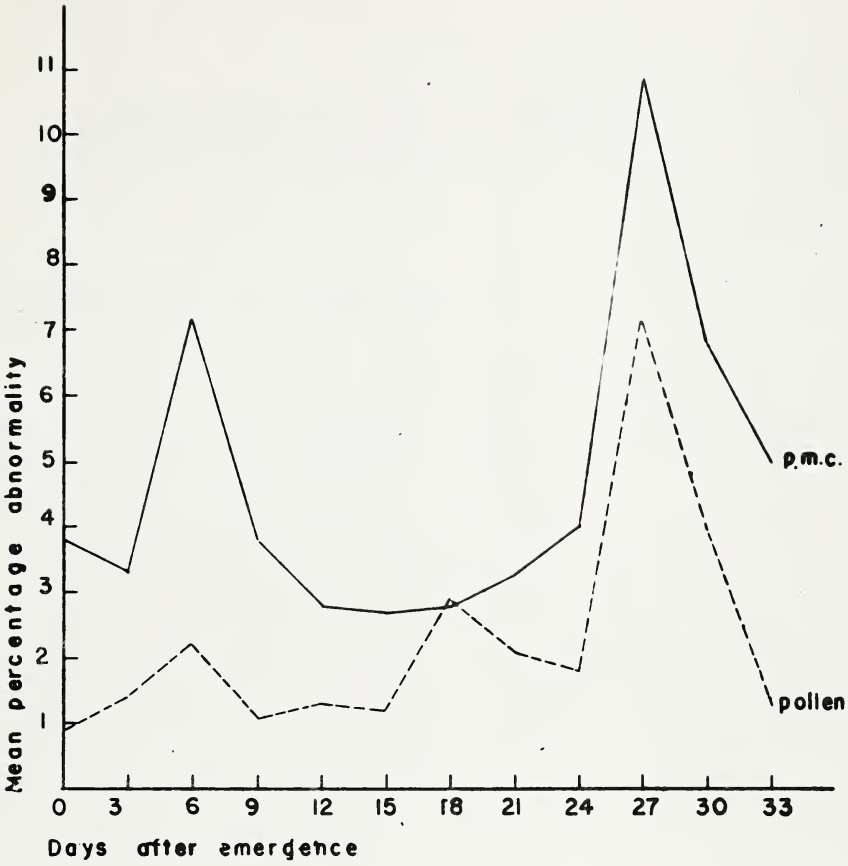

Fig. 26

Abnormality of pollen mother cells and pollen resulting from 16-ounce 2,4-D treatments of barley at different stages of development.

Cytological Data from T1 Generation

Frequencies and Types of Aberration

The results of the cytological analysis of the T1, excluding those from the progeny containing quadrivalents and the centric fragment, are shown in Table 3.

In general, the frequencies of abnormality were similar to those found in treated material. Percentage variations of affected microsporocytes between individual T1 plants was pronounced, the range being from 0.0% to 85.0% in plants studied. In contrast, control material was very regular having only 1.4% abnormal cells, (0.2% lower than that found in field grown checks).

As shown in Table 3, the mean percentage of abnormal pollen mother cells from all plants within certain head rows approximates that found in controls. This would again support conclusions drawn from earlier findings that only certain tillers of treated material were affected. These results from the T1, however, cannot be regarded as conclusive, since too few plants were studied in some of the head rows in which meiosis appeared normal, (Table 3).

Table 3

Frequency of Meiotic Aberrations in T1 Generation

Treated plants	Mean % aberration per head row						No. T1 plants analyzed
	Row 1	Row 2	Row 3	Row 4	Row 5	Row 6	
1	6.8	1.9	15.8	13.6			10
2	21.3	15.4	6.8	23.7	5.3	20.8	18
3	10.7	5.8	0.0	6.7	4.3		9
4	6.6	2.0					2
5	4.6	8.0	18.1				4
6	6.7	2.8	36.2	9.2	3.2		9
7	12.6	4.0	1.0	1.6	10.5		8
8	13.8	6.3	6.5				7
9	14.9	7.6					2
10	9.6	10.8	16.5	5.6	8.4	6.2	16
11	1.4	12.9	3.7	29.8	10.5		11
12	3.9	11.7	9.9	22.3			7
Check	1.4						17

There occurred also, a great deal of variability in percentages of cytological abnormality found within progeny of a single treated tiller. In view of the variation found among different florets within a spike of a treated plant, however, this variability within T1 head rows is not surprising.

Stickiness and fragmentation again were the most frequent aberrations found in the T1 generation, Figs. 4, 6, and 7. Multiploid sporocytes and aneuploidy also occurred consistently, Figs. 8, 9, and 10.

It was concluded from the analysis of treated material that cells with severely sticky and fragmented chromosomes were unable to form functional gametes. The reoccurrence of such aberrations in the T1 generation is further evidence that the chemical or its effects persisted.

This belief was also borne out by a preliminary study of root tip mitoses from germinated seeds of treated plants. Although no fragmentation or stickiness was observed, both c-mitotic pairing and unsatisfactory staining of chromosomes in affected cells were apparent, Fig. 24.

Transmission of Quadrivalents and Centric Fragment

Cytological analysis of 35 T1 plants from the two treated plants containing quadrivalents, revealed that only the progeny of the partially sterile spikes exhibited microsporocytes with rings and chains of four chromosomes. As mentioned earlier, only four of the remaining nine spikes on the first of these treated plants were partially sterile. The second plant produced normal seed set in all remaining spikes.

The proportion of affected and unaffected plants that occurred in each of the four head rows, as well as the chromosomal constitution of the microsporocytes, is shown in Table 4.

Table 4

Chromosomal Constitution of T1 Plants Heterozygous for a Reciprocal Translocation

Tillers of treated plant	T1 plants Without quad.	T1 plants With quad.	Open rings	Zig-Zag. rings	Open chains	Zig-Zag. chains	7^{II}	% quad.	% sterile florets
1	1	3	260	15	32	2	291	51.5	36.1
2	1	1	45	4	6	7	56	52.5	39.2
3	4	1	186	3	6	0	88	68.9	37.2
4	2	2	182	24	24	4	140	62.6	33.6
Total	8	7	673	46	68	13	575		
Mean								58.9	36.5

The mean percentage of microsporocytes that were cytologically observed containing rings and chains of four chromosomes was 58.9%, (5.0% less than that found in affected tillers of the parent plant). Moreover, a high frequency of cells with 7^{II} again occurred, in many of which, two of the seven bivalents were observed to be open. Since the proportion of quadrivalents found in the T1 approximates that found in treated material, viable gametes containing the translocation were formed with a relatively high frequency. As would be expected, approximately one-half of the progeny from affected tillers were heterozygous for the translocation.

Cytological examination of T1 plants from the parent with the centric fragment, revealed that it was in only one of 12 plants examined. In this plant, 85.1% of the pollen mother cells were affected. The fact that the fragment was in only one plant indicates that the aberration was transmitted only by the ovule.

CONCLUSIONS

Field applications of 2,4-D even at the lowest concentration used, were found to cause gross chromosomal irregularities in barley. Nevertheless, even in florets where the percentage of microsporocyte abnormality was high, there still would be produced a sufficient amount of normal pollen to affect fertilization. Moreover, in a diploid species such as barley, competition between normal male gametes and those with slight chromosomal unbalance would probably result in almost complete ineffectiveness of the latter. It would therefore be expected that there would occur little if any, transmission of aberrations through the pollen.

There are possibilities, however, that chromosomal unbalance caused by this chemical could be transmitted through the pollen of polyploid species.

In barley, it is believed that if chromosomal disturbances are transmitted as such, it would occur via the female gamete. Nevertheless, it is again doubtful whether gross chromosomal aberrations or unbalance in barley can be transmitted in this way.

The action of 2,4-D upon cells is in some respects similar to that of colchicine, and in other respects similar to that of known mutagens. In causing doubling and c-mitotic effects on chromosomes, it resembles colchicine. In bringing about fragmentation and reciprocal translocations, it behaves more like a mutagen. The fact that many different types of aberrations are found in any one tiller, floret or anther, indicates that, (1) the action of 2,4-D is not specific, and (2) the chemical remains active long after it has been applied.

While some T1 plants appeared to be morphologically affected, further generations will have to be grown to determine whether the effects were the result of a changed genetic constitution as caused by the chemical. In any case, to determine whether gene mutations have been produced will require further generations.

The fact that translocations and centric fragments were found in the relatively small number of plants that could be studied cytologically, is evidence that 2,4-D even in field strength can cause chromosomal aberrations similar to those caused by X-rays and other mutagens. Until it can be proven that the performance and important characteristics of all future progenies of treated plants are unchanged, the use of 2,4-D as a weed spray on pure seed stocks must be considered hazardous.

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