EFFECTS OF A PREGERMINATION PULSE TREATMENT WITH MORPHACTIN ON *PISUM SATIVUM*

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Summary

A 24-hr pregermination application of morphactin to seeds of *P. sativum* was found, particularly at the higher concentrations used (1–30 mg/l), to stimulate primary root elongation but partially inhibit the initiation and growth of laterals. The latter effect was accompanied by the production of undifferentiated outgrowths at the base of the primary root and a shift of the region of lateral root formation towards the tip. Morphactin was also found to suppress apical dominance and the growth and mitotic rates of shoot tips, but these effects, even after a pulse treatment with 30 mg/l, were not permanent since the plants eventually resumed normal growth and flowered.

A working hypothesis is suggested in which the varied effects of morphactin may be explained by an inhibitory effect of morphactin on some aspects of auxin transport or metabolism or both.

I. INTRODUCTION

Morphactins represent a recently developed group of synthetic plant growth regulators which are readily taken up, translocated, and metabolized in plants (Erdmann, Mohr, and Schneider 1967) and are reported to have a wide range of lasting inhibitory effects on plant growth and development (Schneider 1964). They have been reported, for example, to retard germination (Harada 1967; Sankhla and Sankhla 1967), inhibit root and shoot growth, block apical dominance, inhibit tropic responses (Anon. 1965), and to affect in other ways various morphogenetic processes (Mohr, Erdmann, and Schneider 1966; Ringe and von Denffer 1967), although the evidence is generally incomplete and often contradictory. This paper reports on the effects of a pregermination pulse treatment with morphactin IT3456‡ (methyl-2-chloro-9-hydroxyfluorene-(9)-carboxylate or chlorofluorenol methylester, referred to simply as morphactin) on subsequent growth and development in *Pisum sativum*.

II. METHODS

(*a*) *Seed Germination and Seedling Growth*

Seeds of *P. sativum* (cv. Greenfeast) were surface-sterilized for 10 min in a 1% solution of sodium hypochlorite, and then rinsed three times in sterile water, lightly blotted, and transferred in groups of 20 to sterile 9-cm glass Petri dishes containing 20 ml of water or test

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solution. After 24 hr the seeds were removed, rinsed again in sterile water, and placed on thin foam plastic pads, moistened with 15 ml of water, in new sterile glass Petri dishes. They were then left for 48 hr until the roots were about 15–20 mm long, when 15 from each treatment of 20 seeds were selected for uniformity and transplanted into glass tubes (7 mm internal diameter) expanded at one end to accommodate the seeds. These tubes were then suspended on Perspex racks in 2 litres of inorganic nutrient solution (Heller 1953). After another 48 hr, a further selection of 10 uniform seedlings was made from each treatment, leaving half the original complement for observation. It should be emphasized here that the distinguishing feature of this method was that, apart from the initial 24 hr pregermination pulse treatment, the test seedlings had no further contact with morphactin. All experiments were carried out at 25°C in the dark, observations being made under a 40 W white fluorescent light, covered with a green (growth-neutral) Cinemoid filter (Strand Electric and Engineering Co., London). Measurements were made at 48 hr intervals after removing the glass tubes from the nutrient solution. This technique eliminated direct handling of the seedlings, and ensured periodic aeration of the roots. Plants were removed 10 days after treatment, separated into roots, shoots, and cotyledons, and dried at 65°C to constant weight.

(*b*) *Squash Preparation*

Mitotic counts on the apices of control and treated plants were obtained at 48-hr intervals. Primary root tips (5 mm long) and shoot tips (8–10 cm long) were removed from samples of three plants per treatment, and fixed in 1 part glacial acetic acid and 3 parts absolute ethanol for 24 hr at 4°C. The sections were then transferred to 70% ethanol and stored at 4°C until the squashes could be prepared. Shoot apical domes and root tips 2 mm long were dissected from the fixed material under a binocular microscope and squashes prepared in aceto-orcein (Johansen 1940). The number of cells in different mitotic stages was determined by a systematic scan of the whole preparation, in order to avoid repetition or omission of particular areas. A representative sample of 1000–2000 cells, with the number of cells per field varying generally between 30 and 60, was counted from each squash.

III. Results

(*a*) *Root Growth*

Primary root elongation (Table 1) was in general markedly increased by morphactin in the range 0·1–30 mg/l. In contrast, secondary root initiation was

TABLE 1

EFFECTS OF MORPHACTIN ON ROOT AND SHOOT GROWTH IN *P. SATIVUM*

Average measurements and standard errors were calculated from a total of 10 plants per treatment. Readings taken 10 days after treatment

	Morphactin Concentration (mg/l)						
	0	0·001	0·01	0·1	1·0	10·0	30·0
Primary root length (mm)	105±8	89±14	91±11	128±3	110±13	128±4	150±6
No. of secondary roots per plant	36±2	27± 5	22± 4	29±4	26±2	27±3	15±2
Total dry weight of roots per plant (mg)*	13·4	10·5	8·8	8·5	12·2	10·7	9·4

* Dry weights were determined on the whole sample of 10 plants.

inhibited at all concentrations (Table 1) with the effect becoming particularly marked at 30 mg/l. Added to this was a noticeable change in the distribution

pattern of secondary roots (Fig. 1), with higher morphactin concentrations causing a progressive reduction in peak height and shifting the zone of secondary root formation toward the primary tip, thus indicating a stronger inhibitory effect near the base of the primary root. The overall effect of morphactin on root growth, measured as total dry weight of the root system (Table 1), was slightly inhibitory at all concentrations, but suggested no tendency to concentration dependence.

Higher concentrations of morphactin (10–30 mg/l) also produced gross morphological abnormalities, involving localized zones of intense and apparently uncontrolled meristematic activity, and resulting in large outgrowths of disorganized tissue at the base of the primary root.

Morphactin had no effect on the mitotic rate of primary root meristems.

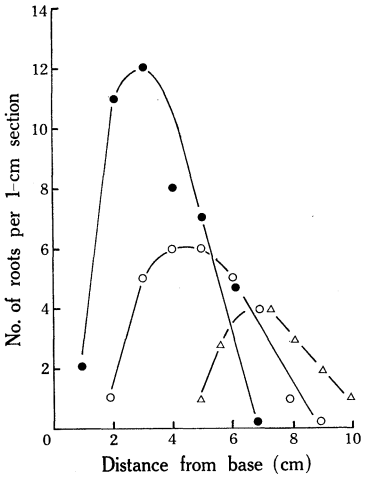

Fig 1.—Effect of morphactin at 10 mg/l (○) and 30 mg/l (△) on the distribution of lateral roots measured 10 days after treatment. ● Control.

(b) Shoot Growth

Although shoot growth, measured by length of the main axis, followed a similar sigmoidal pattern for all treatments throughout the period of observation, higher concentrations of morphactin (1–30 mg/l) caused a gradually increasing and finally complete inhibition of growth at 8–10 days after treatment (Fig. 2). This inhibitory effect was found to be dependent on morphactin concentration (Fig. 3), having a threshold at about 0·1 mg/l and reaching a limiting value of inhibition (measured 10 days after treatment) at 10 mg/l. These results were substantiated by measurements of shoot dry weight (Fig. 4), which indicated the same tendency of morphactin-induced growth inhibition and the same threshold concentration. A subsidiary response to this inhibitory effect on the primary shoot was the release of lateral buds from apical dominance, but the subsequent elongation of the lateral buds was also gradually inhibited.

Although resulting in complete growth inhibition less than 10 days after treatment, the morphactin effect was found to be neither lethal nor permanent. Several seedlings, from seeds treated with the higher morphactin concentrations, when replanted in soil and grown under greenhouse conditions, resumed normal growth between 10 and 12 weeks after treatment and flowered only 3–4 weeks after the control plants.

The effect of morphactin at 10 mg/l on cell division in shoot apices (Fig. 5) shows a time sequence and general inhibitory pattern similar to that indicated earlier by concurrent measurements of shoot length (Fig. 2), suggesting an effect on cell division to be a possible mechanism of morphactin-induced inhibition of shoot growth.

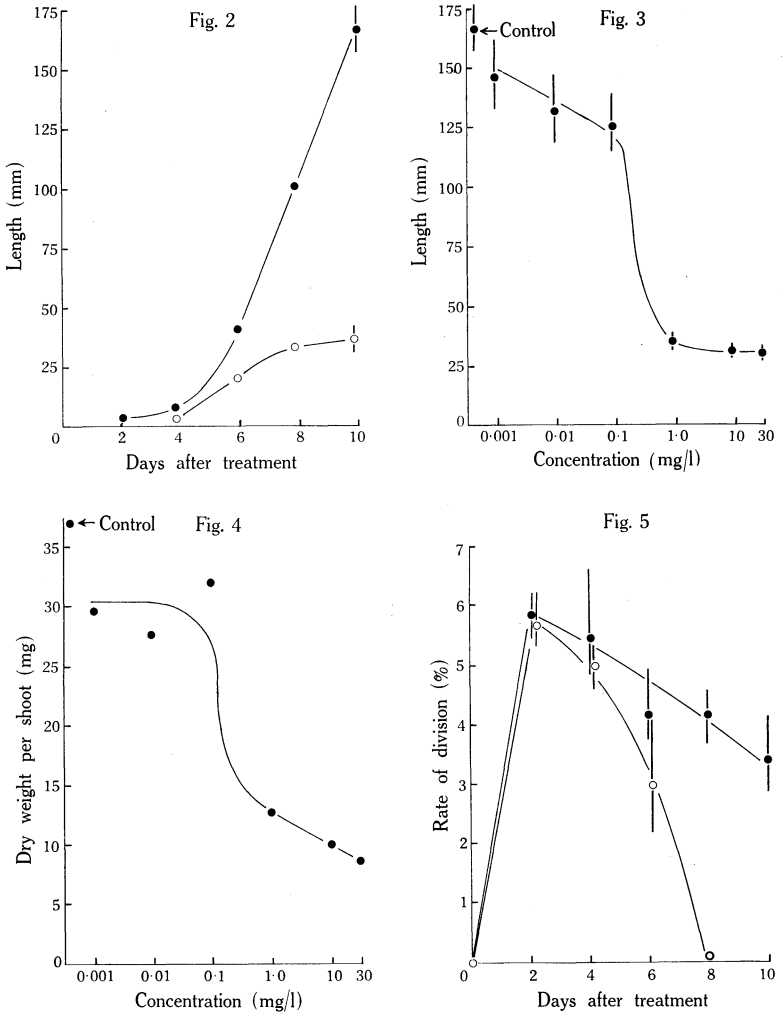

Fig. 2.—Time-dependent effect of morphactin at a concentration of 10 mg/l (○) on shoot growth. ● Control. Vertical lines indicate standard errors.

Fig. 3.—Concentration effect of morphactin on shoot elongation measured 10 days after treatment. Vertical lines indicate standard errors.

Fig. 4.—Concentration effect of morphactin on shoot dry weight. The measurements were averaged from the total weight of 10 shoots harvested 10 days after treatment.

Fig. 5.—Time-dependent effect of morphactin at 10 mg/l (○) on rate of cell divisions in shoot tips. ● Control. Results averaged from three replicate squashes. Vertical lines indicate total variation.

IV. Discussion

A 24-hr pregermination pulse treatment of *P. sativum* with morphactin resulted in stimulation of primary root elongation but a definite inhibition of lateral root initiation, a slight suppression of total root growth (Table 1), and the production of abnormal outgrowths of undifferentiated tissue at the base of the primary root. Added to this was a marked inhibition of shoot growth associated with suppression of apical dominance, and a corresponding inhibitory effect on cell division in the shoot apex. It was found, however, that these inhibitory effects were neither lethal nor permanent under the conditions of application and within the tested range of morphactin concentrations.

The mechanism of morphactin activity has not yet been determined, and it seems likely that such a broad spectrum of physiological activity as that currently ascribed to these compounds may involve more than a single specific mechanism. We shall examine, therefore, in the light of these results, the existing hypotheses for morphactin activity.

Although the generally described symptoms of inhibited internode elongation, reduced nodes, and thicked laminae (Anon. 1965) indicated an inhibitory effect on cell expansion, there is no evidence to support this assumption. Other reports, contradicting the above, indicate enhancement by morphactin of cell elongation in wheat coleoptile sections (Krelle and Libbert 1967, 1968) and intact *Coleus* plants (Krelle and Libbert 1967). Our results may also indicate a stimulatory effect of morphactin on cell elongation in primary pea roots (Table 1) but an inhibitory effect on shoots (Fig. 3), although neither indication has been directly examined.

Morphactins are also known to affect cell division although the nature of their involvement is not yet clear. The production of large meristematic outgrowths on the roots, which have also been reported in other systems (Saniewski, Smolinski, and Pieniazek 1968), suggests a stimulatory effect on cell division. But a direct analysis of mitotic rates in primary root tips from both treated and untreated pea seedlings revealed no significant effect of the morphactin, thus conflicting with a former report of strong inhibitory effects in onion roots (Ringe and von Denffer 1967), although it should be emphasized that the experimental conditions were not the same. Adding to the confusion, the suppression by morphactin of lateral root growth (Table 1, Fig. 1) which has also been previously reported (Firn and Paleg 1968), suggests an inhibitory effect on cell division although no such effect was observed in the primary tip. Moreover, the analysis of division rates in shoot apices reveals a complete mitotic block, induced within 10 days, by morphactin. Thus we cannot, with any confidence, postulate a direct and consistent effect of morphactin on either cell division or elongation, yet there is a possibility that the specific expression of morphactin activity may be dependent on position within the plant.

An alternative and equally acceptable hypothesis involves an indirect influence of morphactin on the processes of both cell division and elongation by modifying the effects of a common endogenous regulator. It has been proposed, for example, that morphactins interact antagonistically with gibberellins (Zeigler, Kohler, and Streitz 1966), although this idea is now in doubt (Tognoni, DeHertogh, and Wittwer 1967). Morphactins have also been shown, however, to counteract the inhibitory effect of

indolylacetic acid (IAA) on lateral shoot growth in pea seedlings and to inhibit transport of [2-^{14}C]IAA along etiolated pea stems (Tognoni 1968). It is possible, therefore, that morphactins may interfere with some aspect of auxin transport or metabolism or both.

Since it is thought that auxin is normally present in pea roots at supraoptimal, inhibitory concentrations (Åberg and Jönsson 1955), exogenous morphactin may interact antagonistically to reduce the effective level of endogenous auxin in the tip region and thus cause elongation of the roots by enhancement of cell expansion. But such a reduction of auxin level in the tip would not account for the inhibition of lateral root growth in the basal regions of the primary root. Changes in the distribution pattern of lateral roots may be attributed to a direct inhibiting effect of a greater morphactin concentration near the base as could result from the pre-germination pulse treatment used in these experiments. However, a plausible alternative is that increasing concentrations of morphactin towards the base may act indirectly by drastically reducing the level of auxin in the basal region. Since it has been established that the rate of cell division and pathway of differentiation are determined (*in vitro*) by the ratio of auxin to cytokinin (Skoog and Miller 1957), and that endogenous cytokinin activity is present in pea seedlings (Zwar and Skoog 1963), it is reasonable to suppose that a progressive reduction by morphactin of auxin activity toward the base of the primary root may produce a corresponding change in the ratio of auxin to cytokinin, and hence suppress the formation of lateral roots. Such an explanation might also account for both the nature and position of meristematic complexes produced by higher morphactin concentrations.

The time-dependent increase in the inhibitory effect of morphactin on shoot growth, and more particularly on mitosis in the shoot apex, may indicate an accumulation in this region of some inhibitor such as morphactin or one of its metabolites (Anon. 1965) or alternatively the gradual destruction of endogenous auxin, which is known to be essential for cell division, or of some other stimulatory substance. The destruction, inactivation, or removal of auxin from the shoot apex may also explain the release of lateral buds from apical dominance while the gradual degradation of the inhibitory substance through normal metabolic processes would account for ultimate abolition of all inhibitory effects as mentioned in Section III. This observation of long-term release has also been reported in other systems (Ringe and von Denffer 1967) and is consistent in this case with the nature of the pre-germination pulse treatment.

Despite implications that the broad spectrum of morphactin-induced physiological effects might involve more than one mechanism of action, it is possible, as suggested above, to postulate a single mechanism of auxin-mediated morphactin activity to account for all of these observations. The precise nature of this interaction, however, cannot yet be defined.

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