ISSN 2349-0837

Duration of Tuber Dormancy in Yam Dioscorea rotundata: Effect of Plant Growth Regulators and its Relationship with Tuber Age

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ABSTRACT

This study seeks to determine the effect of tuber age and plant growth regulators (PGRs) on the duration of D. rotundata tuber dormancy. Tubers of D. rotundata var. Tdr 131, generated from pot grown tissue culture plantlets, were harvested at 75 days after planting (DAP) and at 179 DAP. A set of 30 tubers (untreated control) were observed for date of sprouting. Sets of ten tubers were subjected to one of nine treatments: water (treated control), two concentrations each of 2-chloroethanol (CLE), giberrellic acid (GA3) and CLE/thiourea combinations, and one concentration each of thiourea and ethephon at 183, 214, 269 and 331 DAP. The 50th percentile duration from harvest to sprouting ranged from 152 to 166 d in all controls, and 146 to 184 d in the PGR treatments (corresponding to 26 March and 12 May) depending on PGR. Tuber age at PGR application did not affect the duration from harvest to sprouting but the ethylene analogues: 60 ml l-1 CLE + 20 g l-1 thiourea and 1000 mg l -1ethephon shortened it by 32 d while 1000 mg l-1 GA3 prolonged it by 24 d.

Indexing terms/Keywords

Keywords are your own designated keywords which can be used for easy location of the manuscript using any search engines.

TYPE (METHOD/APPROACH)

Original Research

Council for Innovative Research

Peer Review Research Publishing System

JOURNAL:- Journal of Advances in Biology

Vol. 7, No. 1

editorsjab@gmail.com , editor@cirjab.com

ISSN 2349-0837

INTRODUCTION

Yams are monocotyledonous plants that belong to the family *Dioscoreaceae* and genus *Dioscorea.* They produce edible tubers that have strong agricultural and economic importance in Africa, Asia and South America. It is a crop of prestige particularly in West Africa (Coursey 1967; Martin and Sadik 1977). In West Africa, it is eaten in various forms including boiled, fried and pounded where it serves as a major source of carbohydrate. Millions of people can derive their daily requirements of vitamin C and amino acid and carbohydrate by consuming up to 1 kg of yam per day (Coursey, 1965). Thus, its potential to contribute to the alleviation of hunger and poverty in Africa (IITA, 1978) is generating renewed research interest in yam improvement.

Yams are clonally propagated by tubers or tuber pieces. In West Africa, planting is traditionally done by the start of the growing/ rainy season (Feb. –April depending on agroecology). Harvesting is mostly done in November or December, which coincides with the end of the growing season/ onset of vine and leaf senescence (Onwueme 1975; Passam 1982). Sometimes, tuber harvest is done during the minor harvest season; in August, in order to celebrate the new yam festival. The planting season coincides with the onset of the visible sign of the release of dormancy, which is defined as the appearance of shoot bud (ASB) or sprout on the surface of the tuber (Onwueme 1975; Ile, 2004). In contrast to potatoes (*Solanum tuberosum*), yams have no discernable shoot apical meristems, 'eyes' or external shoot buds in the dormant state, in (Lawton and Lawton 1969; Onwueme 1973; Ile *et al.* 2006). Thus, the duration from planting to main harvest is about 270 days after planting (DAP). When tuber harvest is done during the minor harvest season, the duration from planting to harvest is about 180 DAP. The tubers harvested at the end of the growing season or at the minor season remain dormant for up to 90 or 150 d respectively, even if planted in favourable conditions. Sometimes, some scanty sprouting may be found at about 60 DAP (in January) for some early maturing varieties, stored in favourable conditions. This balancing out of the growth duration and the storage period of tubers is possible an inherent mechanism in tubers that ensures that tubers make daily process towards sprouting with sprouting occurring at the same time of the year (Ile, 2004; Hamadina, 2011). Thus yam tubers express very long period of dormancy. While much is known about the control of dormancy in potato, and seed and bud dormancy generally, knowledge of the mechanism of yam tuber dormancy and how to control it artificially is still rudimentary (Suttle 1996; Craufurd *et al.* 2001; Ile *et al*. 2006). Some factors that determine the duration of dormancy include: varietal/specie differences, age of planting material, tuber age at harvest, post harvest storage conditions, cultural practices and ecology (IITA, 1971; Passam, 1982).

Plant growth regulators (PGRs) such as analogues or antagonists of ethylene, gibberellic acid and abscisic acid (ABA), are known to affect virtually all aspects of plant development (Davies 1987), including tuber dormancy (Suttle 1998 Claassens and Vreugdenhil, 2000). In yams and potato, early physiological studies have shown a strong correlation between the release of dormancy and increase in endogenous ethylene and glutathione (Guthrie, 1940; Campbell *et al.*, 1962a; Okazawa, 1974; Rylski, 1974; Osagie, 1992; Wellington and Ahmad, 1993), and decrease in content of gibberellic acid, phenols and other growth inhibiting substances such as ABA (Park *et al.,* 2001; Ile 2004). Consequently, many attempts have been made to artificially alter the duration of whole yam tuber dormancy using exogenously applied PGRs. It is clear from the reviews that PGRs can affect (prolong or shorten) the duration of dormancy by up to 10 d in *D. rotundata* and 48 d in some other spp. This effect is not hugh enough. Moreover, the effect of PGRs has been inconsistent, *i.e*., PGR may shorten the duration of dormancy/ or duration to ASB in a variety on one study and prolong it on another (Barker *et al.,* 1999). Campbell *et al*. (1962b), Okagami (1979), IITA (1979) Girardin *et al*. (1998); Shiwachi *et al*. (2003) have attributed the inconsistent effect of PGRs on duration to sprouting to differences in the age of tubers at the time of PGR application but no systematic studies have been conducted to verify this claim. Consequently, there is a need to identify effective PGRs that can constituently shorten the duration of dormancy in *D. rotundata* is important. Therefore, the objectives of this study were is to determine: (1. the effect of purported dormancy shortening ethylene based PGRs; 2-chloroethanol, thiourea, ethephon and their recommended combinations, on the duration of dormancy in *D. rotundata* and (2. the relationship between tuber age and the efficacy of PGRs on the duration to sprouting in *D. rotundata*.

MATERIALS AND METHODS

The variety TDr 131 was chosen for this study because of its long tuber dormancy. Field experiments showed that tubers of this variety begin to sprout by late February to early March when harvested in November or December of the previous year. Since differences in timing of vine emergence, in the field, has been shown to lead to difference in timing of tuber production and hence differences in physiological ages of tubers, the whole tubers used in this experiment were generated from plantlets of similar age.

Plantlet propagation

Plantlets were produced at the International Institute of Tropical Agriculture, (IITA), Ibadan, Nigeria. To limit genotypic differences, plantlets were derived from *D. rotundata* var 'Abi' (Tdr 131) apical shoot meristem and cultured as described by Ng (1984). They were multiplied by regular sub-culturing in yam regeneration media (Ng, 1984; 1986) and grown under uniform conditions (Ng, 1984; 1986).

Production of tubers and growing conditions in the glasshouse

Three plantlets were transplanted into each 25 cm pot. Each pot contained a mixture of sand: gravel: vermiculite and loam-less compost in a ratio of 4:2:4:1 (v/v), as well as 3 g l^{-1} Osmocote Plus fertilizer. Osmocote Plus is a slow (90 to 120 d) release fertilizer containing 15 N + 11 P_2O_5 + 13 K_2O + 2 MgO (Monro Horticulture, Goodwood, West Sussex UK). Planting was done on the 1st May and they grew in a glasshouse at Plant Environment Laboratory (PEL), University of

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Reading. From 21 May, the plants were grown for 147 d under a constant 32 ^{o}C day and 22 ^{o}C night temperature and a weekly change of photoperiod that simulated a June planting date at Ibadan, Oyo State, Nigeria. This means that plants received the longest day length at the start of the study and shortening ones during tuber formation and development. Tuber formation in yam is enhanced by short days. Plants were trained on sticks, to expose the leaves of the twining stem to solar radiation for efficient photosynthesis. Pots were watered regularly as required until 288 day of year (DOY). Thereafter, watering was stopped to allow vines to senesce and artificially get the tubers to mature, i.e., for the epidermis to darken in colour and thicken by way of suberization (Passam *et al.,* 1982) before harvesting is done.

Harvest dates and post-harvest storage condition

Tubers were harvested by 25-26 October, *i.e*. at 179 d after planting when vine senescence had began. Tubers were cleaned, weighed, labelled and a total of 390 tubers were stored in a Saxcil growth cabinet maintained at 28 ^{o}C, 80 % relative humidity and 12 h photoperiod. A white fluorescent tube that provided a photon flux density of 0.017 mmol m^2 s^{-1} was used to provide illumination. The weight of tubers used for this study ranged from 4 to 245 g.

Treatments and observations

The experiment consisted of an untreated control, a control treated in water and eight PGRs treatments (Table 1) with 10 tubers (replicates) per treatment. The ethylene based PGRs and concentrations tested were chosen based on review analysis that they shorten the duration to sprouting in other species of yam or other plants that exhibit dormancy. Because gibberellins have the tendency to prolong the duration of dormancy in some species of yam, two concentrations of gibberellin was tested as check for evaluating the effect of the ethylene based PGRs. The PGRs treatments were applied four times, *i.e.* at 183 (harvest), 214, 269 and 331 DAP during storage. On each treatment date, 90 tubers were randomly picked from the tuber lot in the growth cabinet, weighed and then randomly placed into nine buckets. Then nine treatments (treatments 2 to 10) were then randomly assigned to the buckets. Tubers were treated by soaking them in aqueous solution of the above chemicals that contained 6-7 drops of the surfactant, Tween 20.

The surface of treated and untreated tubers was observed every 7 d for the appearance of shoot bud (sprouting). Such an appearance marked the end of visible dormancy. To determine the effects of treatments on the duration from treatment to sprouting, the duration to sprouting for individual tubers was calculated as the difference between date of sprouting and date of treatment.

Table 1. Plant growth regulators and concentrations applied to TDr 131 tubers at each treatment time

Treatment	Concentration	Duration of Treatment (mins)	Abbreviation
1. Experimental control	Distilled water	120	Control (H_2O)
2. Gibberellic acid (GA_3)	150 mg l^{-1}	120	GA_3 150
3. Gibberellic acid (GA_3)	1000 mg l^{-1}	120	GA_3 1000
4. 2 -chloroethanol	4 % or 40 ml l^{-1}	5	40 ml l^{-1}CLE
5. 2 -chloroethanol	6 % or 60 ml l^{-1}	5	60 ml l^{-1}CLE
6. Thiourea	2 % or 20 g l^{-1}	60	Thiourea
7. 2 -chloroethanol + thiourea	60 ml l^{-1} + 5 g l^{-1}	5 + 60	60 ml l^{-1}CLE + 5 g l^{-1} Thiourea
8. 2 -chloroethanol + thiourea	60 ml l^{-1} + 20 g l^{-1}	5 + 60	60 ml l^{-1}CLE + 20 g l^{-1} Thiourea
9. Ethephon	1000 mg l^{-1}	5	Ethephon

Data analysis

Data was analysed using survival data analysis run on Statistical Analysis System (SAS, for Windows, Version 8.0). The program estimates the survival function (S(*t*)) and its standard errors for individual tubers in the respective treatments, at the time of sprouting. The program is particularly useful in the 'analysis of data that correspond to the time from a well defined time origin until the occurrence of some particular event' (Collet, 1994). Because such data are often not normally distributed the more common data analytical methods, *i.e.* the analysis of variance, are unsuitable. It is also useful where the type of distribution is unknown and where censored observations are present, *i.e*. not all tubers in a lot sprouted by the

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end of the experiment. In this case, the data analysed was the duration from date of treatment to the date of shoot bud appearance on the surface of the tuber. The survival time of a tuber is censored if it had not sprouted by the end the experiment. Thus, the exact survival time (S) of such a tuber is unknown but assigned a time (*t*), which is at least as long as the duration of the experiment (Collet, 1994).

In other to make a more conclusive statement about the test hypothesis, the Cox Proportional Hazards Regression (PHREG) procedure was conducted. This is essentially a regression analysis suitable for survival data analysis. It fits a model to the pooled data (weighed scores of the 50th percentile values for all treatments per treatment time) using the Maximum Likelihood Ratio method, and measures the likelihood of the occurrence of sprouting in one treatment relative to the control in the global model. For details see Lee (1992) and SAS version 8.0.

RESULTS

In the untreated control, *i.e.* tubers not soaked in water nor PGR, the 50th percentile duration from harvest (at 179 DAP) to sprouting was 156d, which corresponds to about 4 April of the following year.

Effects of PGRs on duration to ASB

In the experimental control (in water), the 50th percentile duration from treatment at 183 DAP to sprouting was 154 d. This duration was considerably shorter with later treatment dates (Figure 1), suggesting that tubers were naturally less dormant by the next treatment date. In all PGR treatments, the 50th percentile duration from date of treatment to sprouting also declined with later treatment dates (Figure 2).

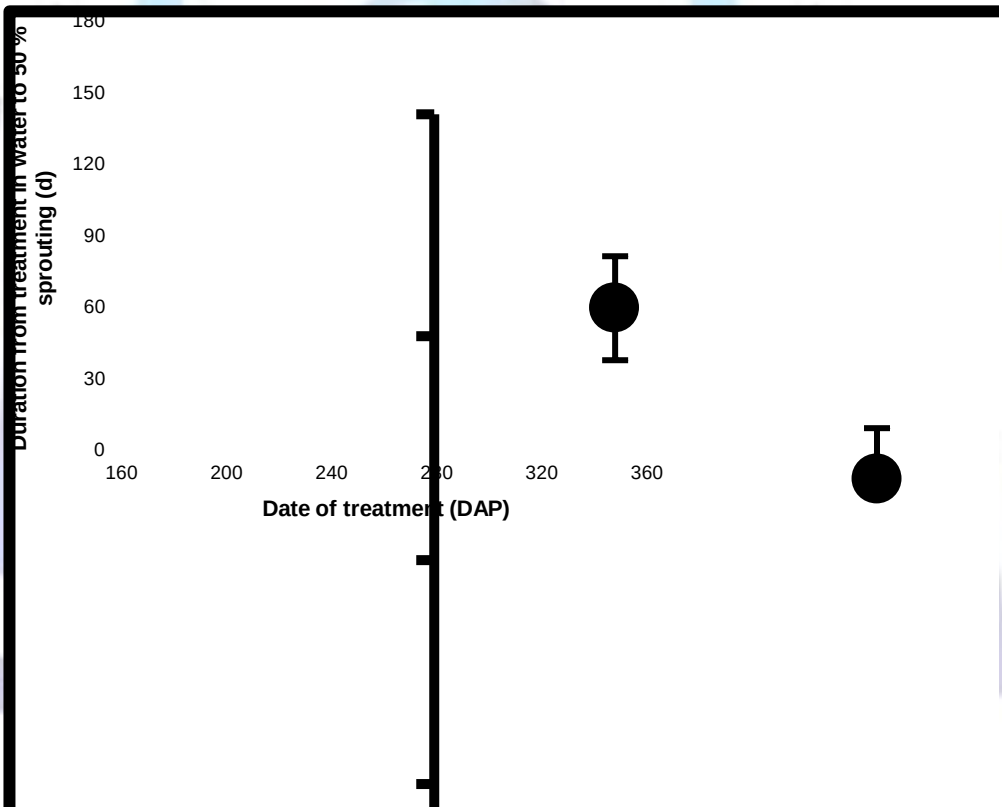

Figure 1. The natural decline in duration to sprouting with every delay in soak in water.

(error bars represent the upper and lower confidence intervals at 95 % confidence coefficient)

Following tuber treatment in PGRs at 183 DAP, the 50th percentile duration from treatment in 1000 mg l^{-1} GA_3 to sprouting was 24 d longer than that of the control (Figure 2a), indicating that the rate towards sprouting was slower in 1000 mg l^{-1} GA_3 than in the control (H_2O). Among the ethylene analogues the combination of CLE and Thiourea, and Ethephon tended to lead to faster rates (up to 10d and 7d respectively) towards sprouting compared to control (H_2O). The effect of a low concentration of GA_3 (150 mg l^{-1}) was similar to that of a low concentration of CLE + Thiourea. Thus low concentration of Ga lead to early sprouting.

Following tuber treatment in PGRs at 214 DAP, a higher concentration of CLE (60 ml l^{-1}CLE), other ethylene analogues except 40 ml l^{-1}CLE, and a low concentration of GA_3 significantly shortened (by up to 32 d in ethephon) the 50th percentile duration from treatment to sprouting (Figure 2b). At the third treatment date, *i.e.* at 269 DAP, both concentrations of GA_3 prolonged the duration to sprouting while the ethylene analogues, particularly 60 ml l^{-1}CLE + 20 g l^{-1} Thiourea, tended to shorten and synchronize the duration to sprouting (Figure 2c). At the last treatment date, *i.e*. at 331 DAP; when 50 %

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tubers were already sprouting but de-sprouted before PGR application, tuber treatment in 1000 mg l^{-1} GA_3 prolonged the duration to sprouting by 7 d when compared with the control (Figure 2d). In contrast, the ethylene analogues, particularly 60 ml l^{-1}CLE + 20 g l^{-1} Thiourea, shortened the duration from treatment to sprouting.

Thus, most ethylene analogues, particularly a combination of high concentrations of CLE and thiourea (60 ml l^{-1}CLE + 20 g l^{-1} Thiourea), and ethephon, can shorten the duration from treatment to sprouting by up to 32 d. In contrast, 1000 mg l^{-1} GA_3 prolong the duration from treatment to sprouting when it is effective by up to 24 d.

Effects of tuber age on the efficacy of PGRs

Tuber age at the date of treatment in PGR affects the efficacy of PGRS. Plant growth regulators were more effective when applied on or before 214 DAP than later (Table 2). The hazard ratio statistics at 183 DAP showed that, 60 ml l^{-1} 2-chloroethanol + 20 g l^{-1} thiourea was 3 times more likely to cause sprouting than the control while 1000 mg l^{-1}gibberellic acid was 0.3 times less likely to lead to sprouting compared to the control. By 214 DAP, the likelihood of all PGRs to cause sprouting increased considerably with ethephon being about 13 times less likely to lead to sprouting compared to the control.

Thus, most ethylene analogues particularly a combination of high concentrations of CLE and thiourea (60 ml l^{-1}CLE + 20 g l^{-1} Thiourea), and ethephon, consistently shortens and synchronise the duration to sprouting (Table 3). In contrast, 1000 mg l^{-1} GA_3 consistently prolongs the duration from treatment to sprouting when it is effective. On the other hand, a low concentration of GA_3 elicits responses similar to ethylene analogues only if applied before 214 DAP. However, whether tubers are treated in water or PGRs sprouting occurred between 149 and 180 days after harvest, which corresponds to the main planting season of yam (end February to 9 April).

Table 2. Duration from date of harvest (at 179 DAP) to sprouting in D. rotundata tubers treated in

Treatment	Concentration	Timing of 50 % (median) sprouting tubers (in d from harvest to sprouting)			
		182 DAP	218 DAP	269 DAP	331 DAP
		Control			
Water		159	166	152	152
		Gibberellic acid			
Gibberellic acid	150 mg l^{-1}	156	149	166	156
	1000 mg l^{-1}	184	166	159	180
		Ethylene analogues			
2-chloroethanol	40 ml l^{-1}	159	159	152	159
2-chloroethanol	60 ml l^{-1}	152	159	159	159
2% Thiourea	20 g l^{-1}	166	149	159	170
6% 2-chloroethanol + 0.5%Thiourea	60 ml l^{-1} + 5 g l^{-1}	156	156	156	152
6% 2-chloroethanol + 2%Thiourea	60 ml l^{-1} + 20 g l^{-1}	149	152	146	156
Ethephon	1 g l^{-1}	153	146	156	156

n=40 tubers/treatment

DISCUSSION

Plant growth regulators affect many developmental processes of plants including dormancy and exogenous application of PGRs have led to slightly early sprouting dates (Craufurd et al 2001; Ile, 2004). Also, anatomical study has shown that gibberellic acid and the ethylene related analogue 2-chloroethanol can prolong and hasten respectively the progress of internal shoot bud development (Ile *et al.*, 2006). However, the effect of PGRs on the duration of whole *D. rotundata* tuber dormancy is minimal and fraught with inconsistencies. This paper reports a systematic study to elucidate the relationship between the age of tubers and the efficacy of PGRs in the control of the duration to sprouting. It also identifies PGRs that significantly shorten the duration to sprouting in *D. rotundata*.

This study has shown that ethylene analogues, particularly a combination of high concentrations of CLE and thiourea (60 ml l^{-1}CLE + 20 g l^{-1} Thiourea), and ethephon, shortens and synchronises the duration to sprouting in a consistent manner. Although 60 ml l^{-1}CLE + 20 g l^{-1} Thiourea successfully shortened dormancy in *D. alata* (Cibes and Adsuar, 1966; Martin

and Cabanillas, 1976), its effect on *D. rotundata* has not been previously reported. Also, the degree of effect observed in this study (up to 26 d) is greater than that reported by Martin and Cabanillas (1976) for *D. alata*, and this may be due to differences in species and tuber age at treatment. The much higher effect reported by Cibes and Adsuar (1966) relates to differences in observations. In their work, they had determined the effect of PGR on the duration from treatment to vine emergence, which is certainly a much longer period compared to the duration covered in this study. With Ethephon, the duration to sprouting in *D. rotundata* var TDr 131 was shortened by as much as 33 d. This degree of effect is certainly greater than that reported in the past for *D. rotundata*, indicating that var TDr 131, which is well known to exhibit long dormancy, may be more responsive to ethephon. In other *Dioscorea* spp., a similar effect and degree of effect of ethephon has been reported. These confirm that ethephon certainly shortens duration to sprouting. In contrast, 2-chloroethanol (CLE) alone and thiourea were not very effective at shortening the duration to sprouting. In spite of the fact that 2-chloroethanol and thiourea, are considered as the most effective ethylene related PGRs in yams (Campbell *et al*., 1962a; Martin and Cabanillas, 1976; Kumar *et al.,* 1998) and in potatoes (Rylski *et al.,* 1974; Coleman, 1998), they have been ineffective in all four treatment times in this study and in *D. alata* (Cibes and Adsuar, 1966). This paper therefore suggests that dormancy in the var TDr 131, which is known to exhibit long dormancy, is well responsive to ethephon and that dormancy in *D. rotundata* can be synchronised and shortened with a combination of CLE and Thiourea (60 ml l^{-1}CLE + 20 g l^{-1} Thiourea). The fact that these PGRs are cheap and available makes them suitable for large studies.

The effect of gibberellic acid on yam dormancy has been highly researched. In this study the ability of 1000 mg l^{-1} GA_3 to consistently prolong the duration to sprouting, when effective, is in agreement with the prolonging effects of GA_3 reported in many studies, and suggests that yams tend to respond to GA_3 in a similar way. In addition to providing information on the effects of a high concentration of GA_3 (1000 mg l^{-1}), which has not been reported in *D. rotundata*, this finding implies that the, less expensive and more available GA_3 (as compared to Uniconazole-P or Prohexadione-calcium) can be used at high concentration to prolong dormancy in *D. rotundata.* These properties render GA_3 more suitable than Uniconazole-P or Prohexadione-calcium for prolonging dormancy in large scale. The possibility of using GA_3 in large-scale research has also been reported (Nnodu and Alozie, 1992; Girardin *et al*., 1998). Further, the fact that Uniconazole-P and Prohexadione-calcium have recently been research on (Shiwachi *et al.,* 2003) and their effects were inconsistent in *D. rotundata and alata*; varying even among varieties of a species as well as with tuber age, suggests that they may not be applicable to a wide range of varieties and species. In contrast to 1000 mg l^{-1} GA_3, a low concentration of GA_3 elicited responses similar to ethylene analogues if applied to young tubers. However, it tended to prolong the duration to sprouting in de-sprouted tubers. Previous works have also shown low concentrations (≤ 150 mg l^{-1}) of GA_3 to produce both shortening and prolonging effects (see Table 1). It is observed here that much of the inconsistent effects of GA_3 shown in Table 1 relate to the effects of low concentrations of GA_3, which may in turn be tuber be related to differences in the age of tubers used in those studies. In the future, the use of high concentrations of GA_3 may produce more consistent prolonging effect.

In spite of the consistent effect (shortening or prolonging) of PGRs when they are effective, the fact that most ethylene analogues did not significantly shorten the duration to sprouting at the last two treatment dates (*i.e.* at 269 and 331 DAP) and 1000 mg l^{-1} GA_3 was not effective at the intermediate ages (*i.e.* 214 and 269 DAP) indicate a role of tuber age on the efficacy PGRs. Wickham *et al.* (1984a) has also found GA_3 to be most effect just after harvest (though tuber age was not clearly define) and in de-sprouted tubers. In view of the experimental considerations taken in this study, these findings clearly confirms earlier suggestions of a role of tuber age (Okagami, 1979; Girardin *et al*., 1998 and Shiwachi *et al.* 2003). Nevertheless, it raises the questions: why were these PGRs not effective on some dates, why were the ethylene analogues most effective at 214 DAP and why was 1000 mg l^{-1} GA_3 more effective just after harvest at 183 DAP and why did 150 mg l^{-1} GA_3 shorten and prolong the durations to sprouting in the first half (183 and 214 DAP) and second half (269 and 331 DAP) respectively.

The bases for a role of tuber age are still not clear. However, it is proposed here that the tubers treated at the first two treatment times (183 and 214 DAP) are within the Phase 2 of dormancy proposed by Ile (2004), while the last two dates (269 and particularly 331 DAP) represented the start of Phase 3 and Phase 3 respectively. This proposal was reached based on the fact that the results showed that the duration from whole tuber treatment at 183 DAP, for example, to sprouting was at least 146 d, and Ile et al. (2006) had shown that the first signs of a tuber germinating meristem (TGM) (mark of end of Phase 1) was seen in the first 90 days after harvest at 179 DAP (*i.e.* at 269 DAP). This implied therefore that the duration from a treatment at 183 DAP to sprouting comprised of three phases of dormancy (Phases 1 to 3). Similarly, the duration from treatment at 214 DAP to sprouting was made up of all three Phases of dormancy. Thus, because tubers were not sprouting by 183 or 214 DAP in this study and in the study in Ile (2004) and no TGM was observed at these dates (Ile, 2004), the tubers treated in PGRs at this date are proposed to be in Phase 1 of dormancy. In addition, because tubers were not sprouting by 269 DAP in this study and in the study reported by Ile et al., (2006) but yet a TGM was observed at this date (Ile, 2004), the tubers treated in PGRs at this date are proposed to be in Phase 2 of dormancy. Finally, because sprouting loci and sprouts were observed by 331 DAP in this study, the tubers treated at this date are proposed to be in Phase 3 of dormancy. If this is true and, following the definitions of the phases of dormancy and the potential mechanisms controlling them as proposed by Ile et al., (2006), then the PGRs tested here should have had no effects at 183 and 214 DAP.

Because PGRs shortened the duration to sprouting by only 7 to 32 d in this study as well as in the literature, it is here proposed that although treatments were applied to whole tubers during Phase 1 of dormancy, the PGRs did not have any effect on dormancy until after the end of Phase 1. As clearly stated by Ile (2004), if Phase 1 had been affected then a much more drastic effect on duration to sprouting would have been observed, *i.e*. 'the observation of sprouting at a time that is prior to 269 DAP or at any date between tuber initiation and 203 d after initiation Ile (2004).

These suggestions may thus be implying that PGRs remain in tuber tissues after being absorbed awaiting the attainment of a ' state of readiness' for growth before they can act to influence sprouting. Long ago Passam (1977) had also suggested that PGRs that are applied to whole dormant tubers do not influence dormancy until some time later. Although he had suggested that PGRs become effective on whole tuber dormancy only after the emergence of a sprout(s), this study suggests that they become effective after the end of Phase 1. Therefore, at the whole tuber level, the degree of effect of a PGR would depend on the availability/ concentration of the PGR at the meristematic layer by the end of Phase 1and start of Phase 2, which in turn may be determined by (1) the date of application, (2) the permeability of the suberised epidermis to PGRs; since Ireland and Passam (1985) have suggested that the tuber epidermis can imposes a barrier to PGR uptake and the epidermis suberises with age, (3) possibly the time taken for PGRs to reach the target, and (5) the level of endogenous PGR(s); since Roberts and Hooley (1988) have shown that level of endogenous PGR(s) can interfere with the sensitivity to applied PGR.

In summary, this study has shown that 1000 mg l^{-1} ethephon and a combination of CLE and Thiourea (60 ml l^{-1}CLE + 20 g l^{-1} Thiourea) can shorten the duration to sprouting by up to 32 d when they are effective. The effects of these PGRs have not been previously reported in *D. rotundata*. In contrast GA_3, particularly at a high concentration, prolonged the duration to sprouting. The effect of 1000 mg l^{-1} GA_3 has not been investigated in *D. rotundata.* In view of the experimental considerations taken in this study *i.e.* use of tubers from plantlets *etc.,* and the clear definition of tuber age at each treatment date, this study has more clearly shown that tuber age moderates the degree of effect of PGRs. Finally, PGRs must shorten the duration to sprouting far more than 33 d if off-season planting is to be achieve. To achieve this, sprouting in whole tubers must occur during Phase 1 of dormancy.

ACKNOWLEDGMENTS

We thank DFID the International Institute of Tropical Agriculture, and The Research Endowment Trust Fund, University of Reading for support to EIH.

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