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Effects of larval host plants on over-wintering preparedness and survival of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

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Abstract

Laboratory colonies of cotton bollworm larvae, *Helicoverpa armigera*, kept at 20 °C under a photoperiod of L:D = 10:14 were fed on five host plants (cotton, corn, kidney bean, tobacco and tomato) and an artificial diet (control) to determine the effects of larval host quality on survival and pupal over-wintering preparedness. A separate experiment showed that diapausing pupae weighed more and contained greater nutrient stores than did non-diapausing pupae. Diapausing pupae reared on different host plants showed significant differences in terms of over-wintering reserve storage, and degree of cold-hardiness (extent of low-molecular-weight substances and SCPs), and survivorship. The more nutrients the host plant had, the more the pupae weighed and the higher the levels of total lipids and glycogen. Body water content was also significantly affected by larval food quality. The mean pupal super-cooling capacities varied significantly from −16.7 to −18.9 °C according to host plants the larvae feed on, and these significantly related to water content, pupal weight, lipid and glycogen content, and the levels of glycerol. Levels of trehalose, glycerol, and inositol, which were mainly low-molecular-weight substances, showed no significant differences among different host plants, except for trehalose. Pupal mortality varied from 39.7% on corn to 3.3% on the artificial diet, which was significantly related to pupal weight, total lipid content, trehalose levels, and super-cooling points. These results suggest that larval food quality can affect survival and influence the over-wintering preparedness of the cotton bollworm.

Keywords: *Helicoverpa armigera*; Host plants; Reserve storage; Low molecular sugars and sugar-alcohols; Over-wintering preparedness; Survival

1. Introduction

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is one of the most serious insect pests in China, Australia and India. This species has a wide range of host plants, including both cultivated and wild species, about one hundred and seventy two species of host plants from 40 families in Australia (Zalucki et al., 1994) and about 200 species from 30 families in China (Xu et al., 1958). Every year, the larvae of this species cause substantial damage to cotton, corn, tomato, kidney bean and other vegetable crops. In China alone, this insect caused several billion RMB losses per year during the 1990s, about 10 billion RMB of which (about 1.25 billion USA dollars) was a direct loss of plants in 1992 (www.stdaily.com/gb/stdaily/2005-10/20/content_444994.htm), before Bt plants had been planted widely. Over most of its range in China, this species produces four or five generations a year and over-winters as a diapausing pupa.

Diapause is an important strategy by which insects avoid unfavorable environmental conditions (Tauber et al., 1986; Danks, 1987). The main factors associated with diapause in insects are photoperiod and temperature (Hodkova and Socha, 1995; Nakai and Takeda, 1995). Other environmental factors, such as food quality (Tzanakakis et al., 1992), have received limited attention. Research on cold hardiness has emphasized the contribution of diapause

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(Tauber et al., 1986; Jiang and Zhang, 1997; Slachta et al., 2002), to the exclusion of possible host plant effects. Nonetheless, food has been shown to be a major factor regulating diapause for a few species of insects (Tauber et al., 1986), influencing the accumulation of energy needed for over-wintering (Zvereva, 2002). Host plant quality affects larval growth rate, the sensitive stage for diapause induction, and cold hardiness (Hunter and McNeil, 1997), which are particularly obvious in polyphagous insects. For example, Zvereva (2002) reported that host plant quality can disturb over-wintering preparedness and thus affect the over-wintering success of the leaf beetle, *Chrysomela lapponica*.

It is well known that insects must accumulate reserves of glycogen before they enter winter diapause (Sakurai et al., 1992). Moreover the accumulated low-molecular-weight sugars and/or sugar-alcohols during over-wintering (Storey and Storey, 1991) are closely correlated with the nutritive quality of host plants (Zvereva, 2002). In this paper, we confirmed that diapausing cotton bollworm pupae weighed more than non-diapausing pupae that had been raised under the same environmental conditions. Clearly, the potential for accumulating reserves is related to the nutritional quality of host plants. Our previous research, which agrees with results reported by Kidd and Orr (2001), suggested that host plants had a significant effect on the development of the immature stage and body weight of pupae of the cotton bollworm (Liu et al., 2004).

Host plants of the cotton bollworm have different nutritive values, which may affect the rate of development of larvae that feed on them and therefore influence the population dynamics of this pest (Ruan and Wu, 2001). The contributions of host plants to developing generations of the cotton bollworm are clear. The suitability of host plants can be ranked as follows: cotton > corn > legume > tobacco > tomato > hot pepper (Liu et al., 2004). To date, the influences of host plants on pupal survival and the cold-hardiness of over-wintering populations remain unclear.

In China, under natural conditions, the fourth or fifth over-wintering generation of the cotton bollworm has a wide range of potential host plants. Changes in cultivation practices have resulted in summer corn being planted on a large scale; corn and concurrently grown cotton, tobacco, and tomato crops supply food of differing quality for the larvae of the over-wintering generation (Lu and Xu, 1998). Differences in the nutritional levels of host plants in the field may affect the over-wintering potential, the over-wintering survival, and the intensity of outbreaks of this pest in the subsequent years. Diapause in the cotton bollworm, *Helicoverpa armigera*, is induced by the short photoperiod and low temperatures of autumn (Li and Xie, 1981; Wu and Guo, 1995) and is terminated by either high or low temperatures (Wu and Guo, 1995). In China, only diapausing pupae can successfully over-winter in the temperate zone. In the field, the cotton bollworm enters winter diapause in October and emerges from this state in late December, after which temperatures below the lowest threshold for pupal development prevent any activity until April of the following year (Jiang and Zhang, 1997). For insect herbivores, growth and development are often intimately linked to host plant quality (Bernays, 1990). Thus differences in the quality of host plants for the cotton bollworm might be expected to influence diapause occurrence and cold-hardiness of over-wintering pupae.

In this study, we reared newly hatched *H. armigera* larvae on five host plants, using artificial diet as a control. We measured the effects of larvae host plants on the over-wintering preparedness of pupae at the beginning of over-wintering (November). This information is essential to the development of a theoretical foundation for managing over-wintering populations and for improving forecasts of the population dynamics of this pest.

2. Materials and methods

2.1. Insects, criteria for determining diapause, and host plants

A laboratory population of *H. armigera* was established by collecting full-grown larvae from cotton plants growing in the suburbs of Changsha, in the province of Hunan. The larvae were reared on an artificial diet with wheat germ as the main component (Wu and Gong, 1997) at 27 °C under a photoperiod of L:D = 14:10 to prevent diapause. Newly hatched larvae were reared in groups until the 3rd instar, after which they were separated in individual glass tubes (2.0 cm dia. × 8.0 cm high) to prevent cannibalism. Mature caterpillars were allowed to pupate in moist soil substrata with a water content of about 7% (soil was heated at temperature 120 °C for 2 h and sifted through sieve with 36 apertures per cm^2). The insects were fed continuously on the artificial diet for 5 generations before testing to reduce the possible influence of the host source (Carrière, 1994).

Two visible morphological characteristics of arrested development, the prolonged presence of pigmented eye spots (Phillips and Newsom, 1966) and the condition of the fat body (Pearson, 1958), were used to determine whether or not pupae were in diapause (Liu et al., 2006).

The following five *H. armigera* host plants which are commonly found in China were used in this study: cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), tomato (*Lycopersicon esculentum* Mill), tobacco (*Nicotiana tobacum* L.), hot pepper (*Capsicun frutescens* L.), and kidney bean (*Phaseolus vulgaris* L.). The seeds used in the experiments were bought from the Chinese Academy of Agricultural Sciences. All plant materials used in the experiments were collected from plants in the field that were grown without pesticides. These plants were fertilized with a controlled release fertilizer and watered as required.

2.2. The over-wintering preparedness of diapausing, and non-diapausing pupae

Larvae were fed on artificial diet at 27 °C under a photoperiod of L:D = 14:10 until the third instar. Thereafter, they were transferred from artificial diet to the fruits of cotton, tobacco, kidney bean, corn, and hot pepper (artificial diet continued to be used as a control), at 25 °C under a photoperiod of L:D = 10:14 to induce winter diapause because third to final instar larvae are most sensitive to diapause induction and one part (50%) of non-diapause pupae can be achieved under these conditions (Li and Xie, 1981). Under these conditions, the duration of last instar varies from 2 to 9 days. The fruits were replaced with fresh ones every other day until pupation. Ten days after pupation, the pupae were checked to see whether diapause had occurred and pupal weight was recorded. Both diapausing and non-diapausing pupae were sampled for total lipid content measurement.

2.3. Inducing diapausing pupae reared on host plants and sampling design

In September 2002, eggs laid on the same days were cut into six pieces and kept in separate glass jars (12 cm dia. × 7.0 cm high) with fresh plant tissue. When eggs hatched, neonates were transferred to glass tubes (one larva per tube to prevent cannibalism) and fed on the young leaves or silk from corn, or on the artificial diet. The leaves or silk were replaced with fresh tissue every other day until the third instar. All six groups of larvae (about 2,000 individuals per group) were reared at 27 °C under a photoperiod of L:D = 14:10 until the third instar. Then all larvae were reared on the fruits of the five host plants (or the artificial diet in the case of the control group), and were maintained at a temperature of 20 °C under photoperiod of L:D = 10:14 to induce winter diapause (Li and Xie, 1981). Diapause was induced in nearly all pupae under these conditions. Mature larvae were allowed to pupate in moist soil as described above. Pupae were examined ten days after pupation to see whether they were in diapause, and then their fresh mass (fw) was weighed. Some pupae from each group were dried for 72 h at 60 °C, and their dry mass (dw) were weighed. Water content (%fw) and dry mass was (dw) calculated as [(fw-dw)/fw] × 100, fw*(1-water content), respectively.

To simulate the over-wintering site of the pest in nature, diapausing pupae were embedded in moist soil in glass tubes (12 cm dia. × 7.0 cm high) and then placed outdoors in bare soil at a depth of 10 cm on 22 October 2002. Air and soil (at a depth of 10 cm) temperature were recorded every day. After one month, on 22 November, over-wintering pupae were dug up to measure over-wintering preparedness. Lipid and glycogen content, super-cooling ability, low-molecular-weight sugars and sugar-alcohols, and mortality were recorded to evaluate the effect of host plants on the over-wintering preparedness of the cotton bollworm.

2.4. Measurement of lipid and glycogen levels

Dried pupae were homogenized and their lipid content extracted with a chloroform-methanol (2:1) solution (Folch et al., 1957). After centrifugation (2600 g for 10 min), the supernatant was removed and the procedure repeated twice. The resulting pellet was dried at 60 °C for 72 h and the lean dry weight (ldw) determined. Levels of lipid content (μg/mg DW) and lipid weight (mg) per individual were calculated using the formula: [(dw–ldw)/(dw/1000)] and dw–ldw.

Fresh pupae were homogenized with 2 ml of 70% ethanol and centrifuged (2600 g for 10 min). Pooled supernatants from two replications of this procedure were discarded and the remaining pellet was used to isolate glycogen according to the method described in Ohtsu et al. (1992). Two ml of 10% (v/v) trichloroacetic acid was added to the residue. The mixture was boiled in water for 15 min and then cooled and centrifuged at 3000 g for 15 min. The supernatant was used to measure glycogen levels. Glycogen was determined by the phenol and sulfuric acid method (Dubois et al., 1956). Absorbance was determined at 490 nm on a spectrophotometer (DU650, Beckman, CO, USA). The results were expressed in mg glycogen g/dw and mg glycogen/individual using a calibration curve obtained by measuring glycogen standards in seven concentrations ranging from 0.025 to 0.5 mg/ml.

2.5. Measurement of super-cooling points (SCPs)

In each host population, the SCPs of 15 healthy pupae were measured. An individual pupa was fixed to a thermocouple attached to an automatic recorder (uR100, Model 4152, Yokogawa Electric Co., Seoul, Korea) via a bridge. The thermocouple with the pupa was lowered into a freezing chamber held at −25 °C; the insect decreased its body temperature at the rate of 1 °C/min, and the decreasing body temperatures were measured. The SCP was taken to be the temperature recorded by the thermocouple just before the increase in temperature caused by the emission of the latent heat of crystallization.

2.6. Measurement of low-molecular-weight sugars and sugar-alcohols

Low-molecular-weight cryoprotectants were determined by capillary gas chromatography as their *o*-methtloxime trimethsilyl derivatives, following the method described by Kostal and Simek (1996). In brief, 10 μl haemolymph per individual was homogenized with 0.4 ml of 70% (v/v) ethanol containing 10 μg of erythritol (an internal standard) in an Eppendorf tube which had been rinsed with 0.2 ml of 70% ethanol. After centrifugation at 10,000 *g* for 5 min, the supernatant was removed and the process

repeated. The pooled supernatant was stored at −20 °C. Before analysis, the samples were evaporated to dryness under a stream of nitrogen at 40 °C in a derivatization vial. Twenty-five microliters of dimethylformamide and 25 μl of *o*-methylhydroxylamine in pyridine (200 mg/ml) were added to the residue for oximation and the sample was then heated at 70 °C for 15 min. Silylation was accomplished by adding 75 μl of dimethylformamide and 30 μltrimethylsilylimidazol to the reaction mixture which was further heated to 80 °C for 15 min. After the desired derivatives were re-extracted into isooctane using 2 × 75 μl of the solvent, 1 μl aliquot was injected into a chromatograph (Pye Unicam 204 GC) equipped with a splitless capillary injector, a flame ionization detector, and an HP 3394 integrator. Separation and quantification of sugars and polyols were achieved on a 30 × 0.25 mm i.d. DB-5 fused silica capillary column (J & W Scientific Inc.). The temperature program was held at 3 min at 120 °C, then increased to 12 °C/min to 280 °C, followed by a 40 min hold. Nitrogen (50 cm/s) was used as a carrier gas. The identification of the components was established against authentic standards.

2.7. Determination of pupal mortality

Larvae reared on tomato had such high mortality that insufficient pupae were available to determine the pupal mortality in this study. About sixty over-wintering pupae from each of the other groups were dug up and kept at 20 °C for 2 days to encourage them to emerge from diapause. Each pupa was then classified as dead or alive on the basis of the presence or absence of abdominal movement in response to stimulation.

2.8. Statistical analysis

Statistical analysis of only diapausing pupae data among host plants for pupal weight, body water content, SCP, lipid and glycogen content, and low-molecular-weight substances such as trehalose, glycerol, and inositol in *H. armigera* over-wintering pupae was performed by SPSS (1999) with one-way analysis of variance (ANOVA), and the means were separated with the Duncan multiple range test (Duncan, 1955). To compare non-diapausing and diapausing pupae, two-way ANOVAs (General Liner Model) were conducted with Duncan multiple range test. Pupal mortality was tested by Chi-square. Regression analysis was used to determine the relationship among survival, SCP, and the other characteristics. The percentage data were arcsine transformed before analysis, and untransformed data are presented.

3. Results

3.1. Effects of host plants on over-wintering preparedness of diapausing and non-diapausing pupae

Both fresh and dry mass of diapausing pupae were much heavier than the fresh and dry mass of non-diapausing pupae, and the diapausing pupae contained more lipids (mg/pupa) than did non-diapausing pupae (Table 1), which showed that larvae destined for diapause accumulate and store energy for over-wintering. The pupal weight and lipid gained, in both non-diapausing and diapausing pupae, varied significantly on different host plants (Table 1).

Table 1
Pupal weight and lipid content (Mean ± SE) between diapausing and non-diapausing *H. armigera* fed on different host plants*

	Cotton	Corn	Kidney bean	Tobacco	Hot pepper	Artificial diet
Fresh pupal weight (mg)						
Diapausing	294.6 ± 5.0 (45) b	253.4 ± 8.3 (34) c	295.2 ± 6.67 (29) b	282.0 ± 5.4 (30) b	207.1 ± 5.0 (21) c	356.8 ± 5.4 (46) a
Non-diapausing	256.4 ± 13.3 (6) b	221.0 ± 8.6 (11) c	258.3 ± 8.1 (29) b	256.3 ± 3.9 (41) b	180.4 ± 8.3 (19) d	303.6 ± 7.1 (33) a
P	0.013*	0.038*	0.001**	0.001**	0.008**	<0.0001**
Dried pupal weight (mg)						
Diapausing	91.7 ± 1.3 (45) b	86.3 ± 2.8 (34) b	88.2 ± 2.42 (29) b	75.0 ± 1.4 (30) c	73.5 ± 1.8 (21) c	108.7 ± 1.6 (46) a
Non-diapausing	79.9 ± 4.2 (6) b	78.9 ± 3.1 (11) b	74.9 ± 1.93 (29) b	66.8 ± 1.0 (41) c	64.3 ± 2.9 (19) c	92.5 ± 2.2 (33) a
P	0.008**	**0.007****	0.001**	0.001**	0.01*	<0.0001**
Total Lipid (mg/pupa)						
Diapausing	33.6 ± 2.1 (8) a	32.9 ± 3.4 (9) a	31.9 ± 2.8 (10) a	24.2 ± 1.1 (20) b	31.0 ± 2.1 (11) a	
Non-diapausing	25.7 ± 2.7 (6) b	32.0 ± 1.7 (6) a	23.6 ± 2.2 (10) bc	18.9 ± 0.9 (20) c	29.3 ± 2.8 (9) ab	
P	0.040*	0.849	0.030*	0.001**	0.625	
Total Lipid (ug/mg DW)						
Diapausing	371.1 ± 14.9 (8) b	387.4 ± 11.7 (9) ab	364.5 ± 16.6 (10) b	312.1 ± 13.7 (20) c	421.8 ± 12.1 (11) a	
Non-diapausing	322.5 ± 27.7 (6) c	397.5 ± 13.2 (6) b	324.1 ± 15.6 (10) c	271.8 ± 10.3 (20) d	450.9 ± 18.6 (8) a	
P	0.124	0.581	0.093	0.001**	0.189	

*Means within row followed by different letters are significantly different at $P \leqslant 0.05$ (Duncan's multiple range test).

Table 2
Over-winter reverse storage of diapausing *H. armigera* fed on different host plants[a]

Host plant	Pupal weight (mg)		Water content[b]	Total lipids		Glycogen	
	Fresh weight	Dry weight	%FW	Weight (mg/individual)	μg/mg DW	Weight (mg/individual)	μg/mg DW
Cotton	270.5±1.4 (696) b	85.4±0.5 (696) c	68.4±1.0 (18) a	33.9±3.9 (17) ab	430.3±31.8 (17)a	9.7±0.9 (5) a	106.8±7.0 (5) a
Corn	228.2±4.3 (109) d	65.8±1.2 (109) d	71.2±0.8(10) ab	22.7±2.0 (10) bcd	370.9±1.15.8 (10)ab	6.5±1.0(3) b	77.9±3.8 (3) ab
Kidney bean	245.0±2.3 (351) c	67.1±0.6 (351) d	72.6±1.4 (16) b	20.0±3.2 (16) cd	301.9±27.4 (16)b	5.3±0.5 (5) b	66.0±6.6 (5) b
Tomato	143.8±6.9 (15) e	46.8±2.2 (15) e	67.4±1.9 (8) a	17.1±1.9 (8) d	374.3±17.0 (8)ab	4.3±1.3 (3) b	82.4±19.4 (3) ab
Tobacco	311.4±4.4 (151)a	98.2±1.4 (151) a	68.5±1.1 (9) a	39.6±6.3 (9) a	353.2±29.6 (9)ab	6.7±1.4 (3) b	70.7±12.2 (3) b
Artificial diet	299.5±2.7 (238) a	91.2±0.8 (238) b	69.5±0.9 (17) ab	31.1±3.7 (14) abc	357.3±17.5 (14)ab	6.5±0.8 (3) b	73.6±6.3 (3) ab
F	128.073	243.343	2.595	4.546	3.117	4.769	2.424
P	<0.0001	<0.0001	0.032	0.001	0.014	0.007	0.081

[a]Means within columns followed by different letters are significantly different at $P \leqslant 0.05$ (Duncan's multiple range test).
[b]Data were arcsine transformed before analysis and untransformed data are shown.

3.2. Over-winter reserve storage for diapausing pupae among different host plants

The pupal weight of the over-wintering population was significantly affected by the larval host plant (Table 2, $F = 128.073$; df = 5, 1554; $P < 0.001$). The fresh weights of the pupae of larvae reared on tobacco and cotton, and on the artificial diet, were significantly more, 311.4, 297.5, 270.5 mg, respectively, than those of larvae reared on kidney bean, corn and tomato (245.0, 228.3 and 143.8 mg, respectively). Similar results were found for dry weights.

Pupal body water varied from 68 to 71% according to the larval host plant, and significant differences between the six groups are shown (Table 2, $F = 2.595$; df = 5, 97; $P < 0.032$).

Pupae's average total lipid contents among the six groups differed significantly (Table 2, $F = 4.546$; df = 5, 68; $P = 0.001$), ranging from 39.6 mg/individual for larvae fed on tobacco to 17.1 mg/individual for those fed on tomato (Table 2). Pupae of larvae reared on tobacco, cotton, and artificial diet contained more lipids than did pupae of larvae reared on corn, kidney bean and tomato. When measured as μg/mg DW, the data showed significant differences among host plants ($F = 3.117$; df = 5, 68; $P = 0.014$), although the differences were much decreased.

Pupal glycogen content also differed significantly between the six groups ($F = 4.769$; df = 5, 16; $P = 0.007$) (Table 2). Pupae of larvae fed on cotton contained more glycogen (9.72 mg/individual) than did those of larvae fed on other host plants. Measured as ug/mg DW, glycogen levels ranged from 106.8 μg/mg dw for larvae fed on cotton to 66.0 μg/mg dw for larvae fed on tobacco.

3.3. SCPs of over-wintering pupae

The SCPs of over-wintering pupae were also significantly affected by host plants ($F = 3.746$; df = 5, 88; $P = 0.005$). The mean SCPs of pupae from larvae reared on cotton, tobacco, and the artificial diet were −18.9, −18.6, and −18.6 °C, respectively, and were much lower than the SCPs of pupae reared on tomato, which was only −16.7 °C (Fig. 1). The relationships of SCPs to other pupal characteristics were analyzed and shown to be dependent on water content, pupal weight, lipid and glycogen content, and the concentration of glycerol (Fig. 2).

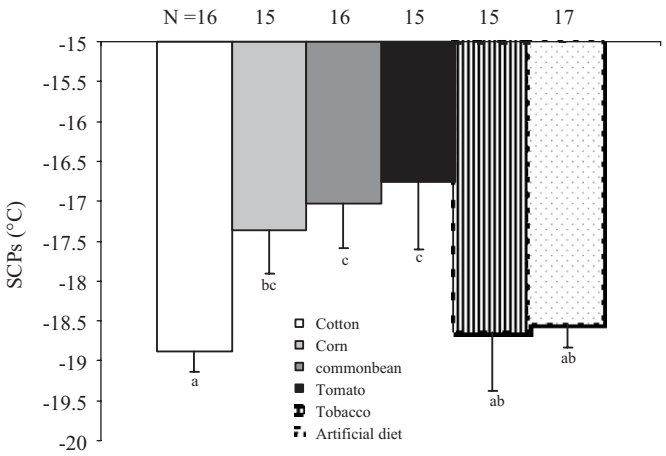

Fig. 1. Super-cooling points of over-wintering pupae of *H. armigera* reared on different host plants. N indicates the number of samples tested. Bars on the column indicate standard error. Different letters below the bars indicate significant differences at $P \leqslant 0.05$ (Duncan's multiple range test).

3.4. Low-molecular-weight substances

Three main low-molecular-weight substances were identified as free sugars and sugar-alcohols in the *H. armigera* over-wintering pupae: trehalose, myo-inositol and glycerol, although some kinds of other polyols were present in haemolymph (Table 3). At the start of over-wintering, only trehalose exhibited significant differences among pupae of larvae fed on different host plants (Table 3, $F = 26.709$; df = 5, 16; $P < 0.001$). Trehalose was the low-molecular-weight substance with the highest concentration (> 20 μg/μl haemolymph); the other two had lower concentrations (< 2 μg/μl haemolymph) (Table 3).

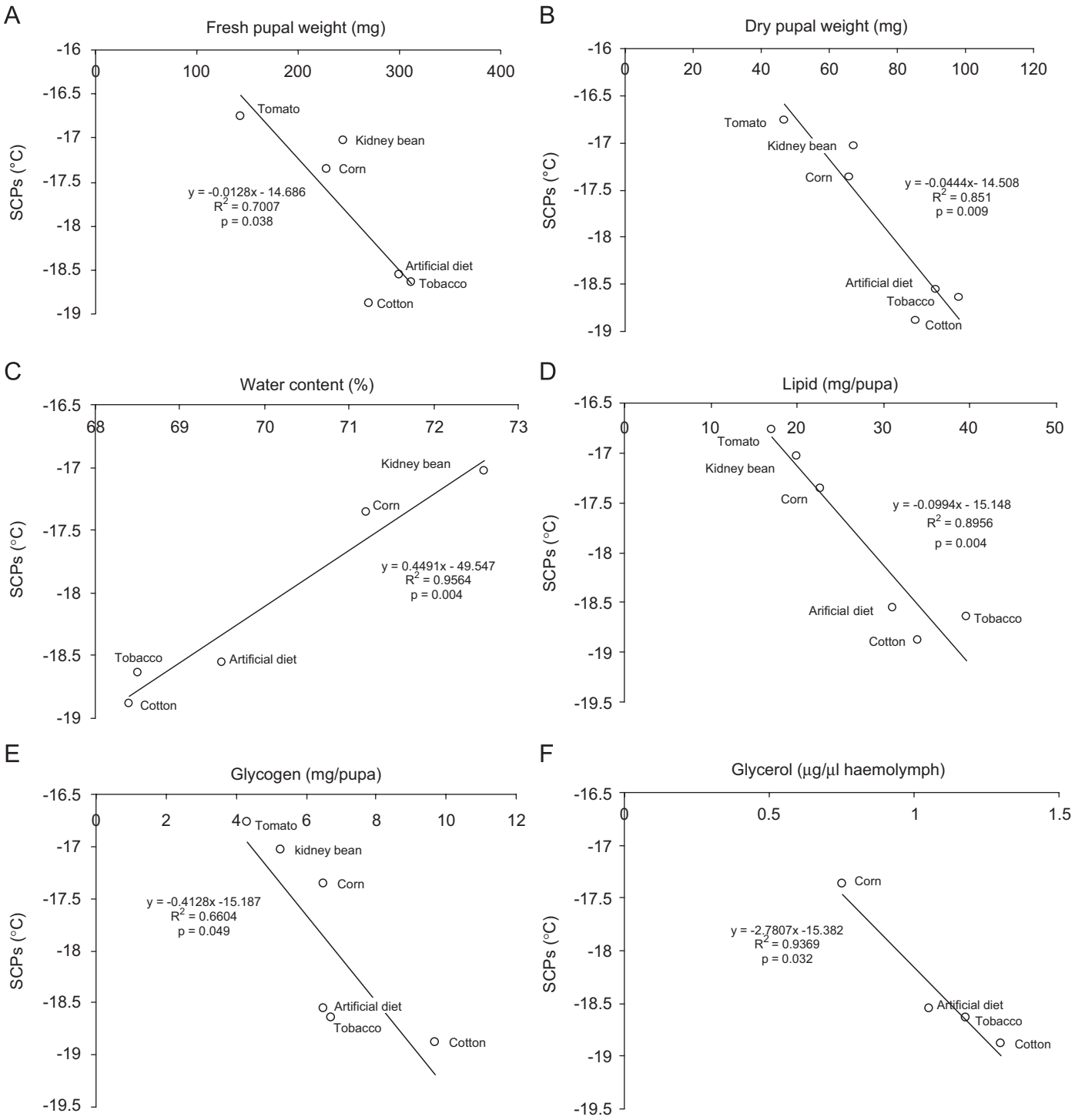

Fig. 2. Regression of SCP on pupal over-wintering characteristics. Each point in the figure represents one kind of host plant as shown in the figure. The equation, R^2, and P are shown in the figure. When $P<0.05$, it means the regression is significant.

3.5. Environmental temperature and pupal survival

Air and soil (at 10 cm) temperatures were recorded daily from 22 October to 22 November. During this period, the minimum soil and air temperatures were about −3 and −10 °C, respectively. On most days, temperatures were around 0 and −5 °C for soil and air, respectively. The ambient temperatures were much higher than the supercooling points.

Pupal mortality among the different groups was significantly different ($\chi^2 = 27.3783$; df = 5; $P<0.0001$) (Fig. 3). The pupal mortality of larvae reared on corn and kidney bean was 39.7 and 26.9%, respectively, and these values were significantly higher than those of larvae reared

Table 3
The concentration (μg/μl haemolymph) (Mean ± SE) of low molecular weight substances of over-wintering pupae from larvae fed on different host plants[a]

Host plant	*n*	Glycerol	Myo-inositol	Trehalose	Glucose	Mannitol	Sorbitol	Dulcitol
Cotton	5	1.30 ± 0.27 (5) a	1.78 ± 0.54 (5) a	37.44 ± 6.26 (5) b	ND	ND	ND	ND
Corn	4	0.75 ± 0.01 (3) a	1.52 ± 0.13 (4) a	154.59 ± 13.03 (4) a	ND	0.13 (1)	0.39 (1)	0.42 (1)
Kidney bean	5	1.49 ± 0.17 (5) a	0.58 ± 0.06 (4) a	36.86 ± 7.26 (5) b	ND	ND	1.60(2)	ND
Tobacco	3	1.85 ± 0.93 (3) a	1.57 ± 0.35 (3) a	38.77 ± 9.76 (3) b	ND	ND	ND	ND
Tomato	3	1.18 ± 0.12 (3) a	1.83 ± 0.22 (3) a	28.33 ± 3.13 (3) b	0.83 (1)	0.34 (1)	ND	0.32 (1)
Artificial	5	1.05 ± 0.30 (5) a	1.24 ± 0.28 (5) a	40.69 ± 5.27 (5) b	ND	ND	ND	ND
F		0.996	2.083	26.709				
P		0.453	0.124	< 0.001				

Means within columns followed by different letters are significantly different at $P \leqslant 0.05$ (Duncan's multiple range test).
[a] *n* is the number of test individuals, values in parenthesis are the number of samples that low-molecular-weight substances are present. ND means non-detected.

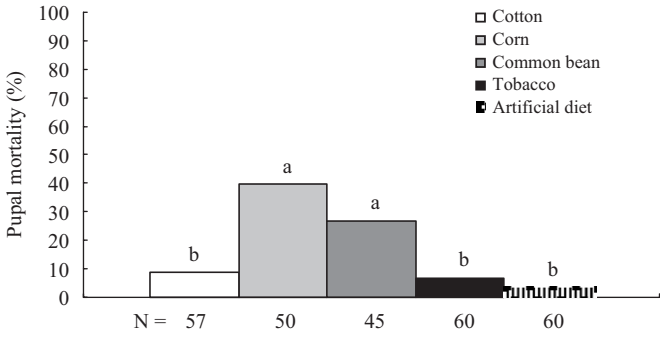

Fig. 3. Pupal mortality of over-wintering *H. armigera* reared on different hosts. N indicates the number of samples tested. Different letters above bars indicate significant differences at $P \leqslant 0.05$ (χ^2 test).

on cotton, tobacco and the artificial diet (8.9, 6.7 and 3.3%, respectively).

The relationships between pupal mortality and over-wintering preparedness (as measured in terms of pupal weight, water content, lipid and glycogen levels, with low-molecular-weight substances and SCPs) were analyzed; survival was significantly related to pupal weight, total lipid contents, the concentration of trehalose, and SCPs(Fig. 4).

4. Discussion

Different host plants contain various nutrient levels, which may affect the ability of *H. armigera* pupae to over-winter. This study showed that for all the tested host plants, diapausing pupae of *H. armigera* are much heavier than non-diapausing pupae; this results is supported by our previous study on summer-diapausing pupae (Liu et al., 2006). Clearly, larvae destined to become diapausing pupae accumulate energy before diapause is induced, confirming that *H. armigera* belongs to the category of insects that needs to accumulate storage for over-winter. Insects' ability to store energy varied according to their larval host plants, such as pupal weight and lipids and glycogen levels. Furthermore, SCPs and levels of low-molecular-weight substances also obviously varied among diapausing pupae according to their larval host plants. The ability of super-cooling was associated with host plants and significantly related with pupal weight, water content, lipid and glycogen, and the concentration of glycerol. Pupal mortality also varied among host plants and was significantly related to pupal weight, lipid content, SCPs, and the concentration of trehalose. The higher the quality of the larval host plants, the better the insect's preparedness for over-wintering, and the higher its chances for survival.

Individuals can accumulate larger amounts of nutrients during pre-winter feeding (Hokkanen, 1993). The larger the pupae, the more energy stored. Large amounts of metabolic reserves, in the form of lipids and glycogen, are typically accumulated prior to diapause (Kostal et al., 1998; Ding et al., 2003). Lipid and glycogen are two major forms of energy reserves whose patterns of use can differ during diapause (Adedokun and Denlinger, 1985). Lipids are the energy source for post-diapause development (Kostal et al., 1998) and probably directly affect post-diapause pupal survival. In our experiments, lipid levels varied when larvae were fed different host plants. The more suitable host plants, such as cotton and tobacco, the more lipids were contained in over-wintering pupae, suggesting stronger over-wintering potential (such as SCP and pupal mortality, see Figs. 2D and 4C, respectively). In our research, we only measured total lipids and did not distinguish triglycerides (major form of energy reserve) from other classes of lipids (phospholipids, sterols, diglycerides, etc.) because these can reflect general responses and functions of host plants. But the detailed responses of each lipid component caused by host plants deserve further study. Glycogen, which serves as the main metabolic fuel during the inactive diapause state, decreases substantially toward the end of diapause (Kostal et al., 1998). Its metabolism is linked to the production of cryoprotectants (Li et al., 2002; Thompson, 2003). In our study, the levels of accumulated glycogen reserve are highly linked to the ability of super-cooling (see Fig. 2E), showing the proof of its importance for over-wintering potential.

Besides accumulation of lipid and glycogen, many insect species accumulate low-molecular-weight substances such as cryprotectants (Salt, 1961). Low-molecular-weight sugars and polyols, including glycerol, trehalose and

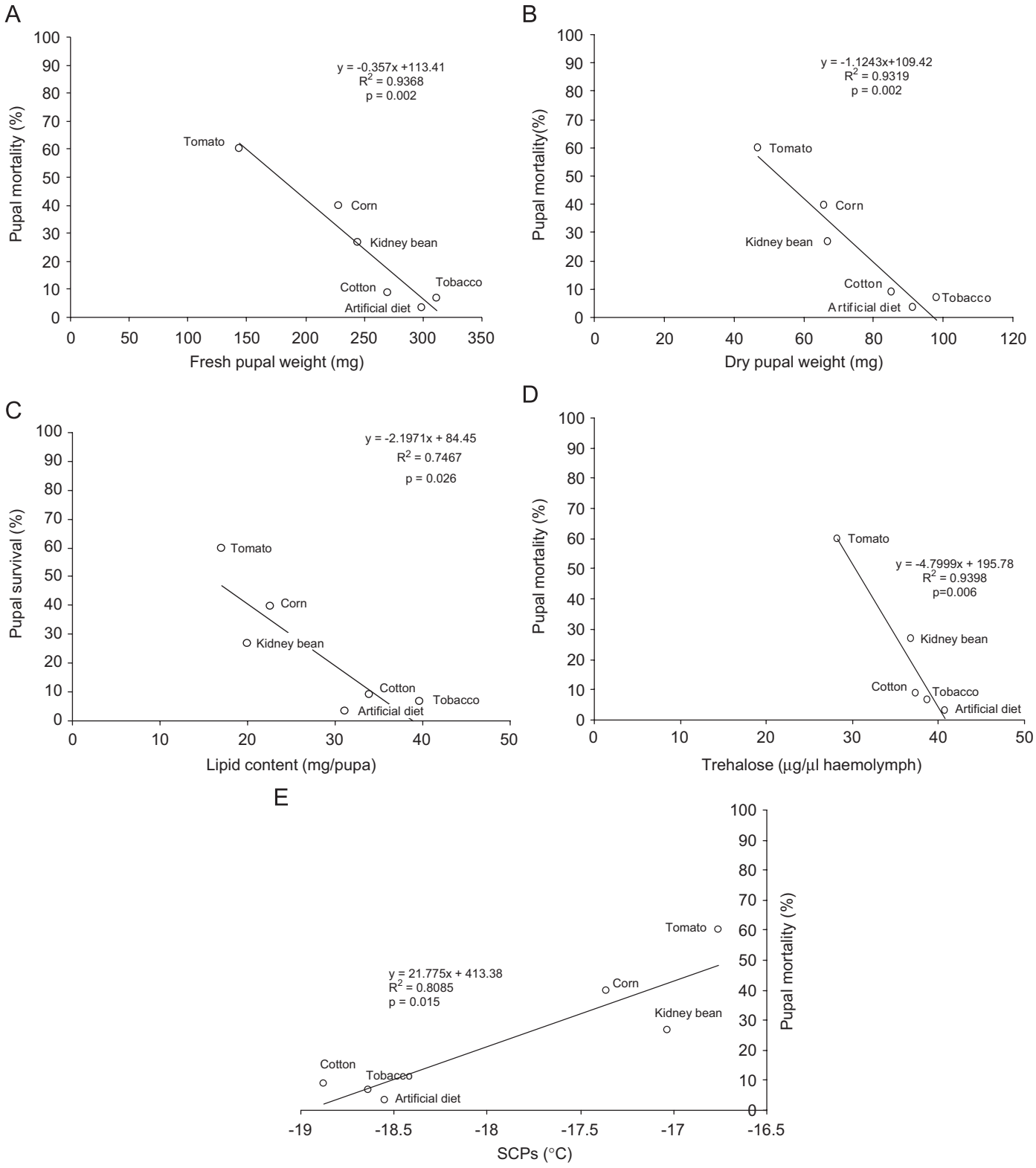

Fig. 4. Regression of pupal mortality on over-wintering preparedness (pupal weight, water content, energy storage, low-molecular-weight substance, and SCPs). Each point in the figure represents one kind of host plant as shown in the figure. The equation, R^2, and P are shown in the figure. When $P<0.05$, it means the regression is significant.

inositol, have been reported as cryprotective agents in many species of insects (Storey and Storey, 1991). In this study, of all low-molecular-weight substances, only trehalose showed significant differences among different host plants. This may be due to warmer conditions during the beginning period of over-wintering, which did not give

much stress to produce much more other low-molecular-weight substances. The concentration of trehalose was very high for pupae reared on corn, which may be caused by the sugar-rich food provided by corn. Low-molecular-weight substances–specifically levels of glycerol and trehalose–play important roles in both SCPs and pupal survival during over-wintering (shown in Fig. 2F and 4D), indicating glycerol is a main super-cooling reagent and trehalose is involved more in metabolism (Munyiri and Ishikawa, 2005).

In many insects, such as the cotton bollworm, SCPs have been used as an index of cold hardiness (Worland, 2005). High super-cooling points can often be attributed to the absence of ice-nucleating agents, an accumulation of cryoprotectant elements, or both (Milonas and Savopoulou-Soultani, 1999). In our experiment, these factors were influenced by larval host plant quality. First, the better the food quality, the lower the water content; low water content can elevate the concentration of cryoprotectant substances and thus enhance cold hardiness (Danks, 2000). We carried out regression analyses between SCPs and water contents and found they both related significantly (Fig. 2C). Second, the strong super-cooling ability among pupae that fed on suitable host plants may be attributed to the presence of lower-molecular-weight substances that were converted from glycogen. These accumulated low-molecular-weight substances act as cryoprotectants to enhance cold hardiness at low temperatures (Li et al., 2002). Moreover, SCPs were significantly related to the concentration of glycerol (Fig. 2F). However, SCPs only set the lower limits of supercooling capacity, and, as such, represent a theoretical lower limit for the survival of insects that cannot tolerate freezing. Many insects show considerable non-freezing mortality at temperatures well above the SCPs (Carrillo et al., 2005), including *H. armiegra*. However, it is unknown whether the differences in SCPs actually represent the differences in cold hardiness at higher sub-zero temperatures.

Over-wintering success during a prolonged winter depends on both environmental factors and internal physiological processes (Han and Bause, 1998). One of the major factors which determine the performance and physiology of herbivorous insects is diet quality (Scriber and Slansky, 1981; Naya et al., 2007). Over-wintering mortality may result from the failure of diapause development, inadequate preparations against the cold, or the exhaustion of energy reserves, all of which can be significantly influenced by pre-winter conditions (Han and Bause, 1998). In our experiment, at the onset of winter, pupal mortality of larvae fed on kidney bean and corn was significantly higher than that of larvae fed on cotton, tobacco and artificial diet (Fig. 3), although, the minimum soil and air temperature was about −3 and −10 °C, respectively, far higher than the SCPs (−17 °C). We attribute this mortality to direct and indirect effects of host plant quality. The direct effects were caused by accumulated plant secondary substances which stressed the pupae. The indirect effects were caused by the over-wintering pupae being susceptible to low temperature as a result of physiological differences. The former was proved in our previous tests (Liu et al., 2004). In general, the larvae can pupate and few pupae die once they have survived to the third instar on artificial diet. However, this is not the case for larvae reared on host plants. Some deaths occurred from third to final instar larvae, even for the pupae which were thought to be caused by accumulated plant secondary substances, indicating that some host plants were not suitable. The indirect effects may be explained by current data. The internal mechanism can be explained by the extent of energy reserves and cold hardiness potential of different host plants, which were confirmed by our regression analysis (Fig. 4). In this study, we have not found that pupal water content affected pupal survival very much, although high body water content reportedly negatively affects cold hardiness and survival in insects (Cannon et al., 1985; Worland, 2005). We did find that larger individuals, which have heavy body mass and a large energy storage capacity, usually have a higher probability of surviving the onset of winter (Fig. 4), supporting the theory of Zvereva (2002). Although, we don't know how exactly host plants affected pupal mortality on low temperatures, the data show that host plants can affect the physiological index and the chances of winter survival. A question that then arises is whether larvae would feed exclusively on plants that are not so suitable. Although *H. armigera* can transfer between Bt and non-Bt cotton, as reported by Men et al. (2005), they don't have many chances to transfer from these main host plants because of the planting structure and area in the field.

In this study, we could not rear enough pupae on tomato to test mortality because of high mortality for larvae fed on fruit in the lab. First, tomato itself is not a good host plant for *H.armigera* larvae, as previous work (Liu et al., 2004) has shown: only 3% of larvae can survive under 27 °C with 14:10 photoperiod. Second, it is rearing problem with tomato fruits. Although we changed the fruit every day, some larvae drowned in rotten tomatos. We only tested insects' over-wintering capability at the beginning of the winter. However, as the over-wintering dynamics of this insect over the whole season are still unclear, further research on the effects of different host plants will no doubt be very informative. Meanwhile, how the host plants affect the occurrence of diapause and larvae behavior before over-wintering is a very interesting project. By understanding how host plants and insects interact, we can better manage the pest and predict population abundance in different host landscapes in the future.

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